Predicting wheat maturity and stay –green parameters by modeling spectral reflectance measurements and their contribution to grain yield under rainfed conditions

Germain Montazeaud¹, Handan Karatogma¹, Ibrahim Ozturk¹, Pierre Roumet², Martin Ecarnot², Jose Crossa³, Emel Ozer⁴, Fatih Ozdemir⁴, Marta S. Lopes^{1,*}

¹CIMMYT, PO Box 39, Emek, Ankara 06511, Turkey

²INRA – UMR AGAP Campus Supagro Montpellier, place Pierre Viala 34060 MONTPELLIER CEDEX 2, France

³CIMMYT, Int. Apdo. Postal 6-641, 06600 Mexico, DF, Mexico

⁴Bahri Dagdas Uluslararası Tarımsal Araştırma Enstitüsü, PK:125 , Karatay, Konya, Turkey

Corresponding author: m.dasilva@cgiar.org

Abstract

The normalized difference vegetation index (NDVI) continues to provide easy and fast methodologies to characterize wheat genetic resources in response to abiotic stresses. This study identifies ways to maximize green leaf area duration during grain filling and develops NDVI models to predict physiological maturity and different stay -green parameters to increase grain yield of rainfed winter wheat under terminal drought. Three wheat populations were evaluated: one containing 240 landraces from Afghanistan, the second with 250 modern lines and varieties, tested for two years under low rainfall conditions in Turkey, and the third with 291 landraces from Central and Western Asia (grown for one year in the same location). The onset of senescence, maximum “greenness”, rate of senescence and residual “greenness” at physiological maturity were estimated using sequential measurements of NDVI and have shown significant correlations with grain yield under low rainfall rainfed conditions. Trade-offs were identified among the different stay -green attributes, e.g. delayed onset of senescence and high maximum “greenness” resulted in accelerated rates of senescence and highest yields and were most evident in the **landrace** populations. It is concluded, that the use of rate of senescence to select for stay – green must be coupled with other stay –green components, e.g. onset of senescence or maximum “greenness” to avoid the effects of the trade-offs on final grain yield. The NDVI decay curves (using the last three NDVI measurements up to maturity) were used to estimate days to maturity using the NDVI decay during the senescence period and days to heading. A training and testing set (20 and 80% of each population, respectively) were used for calibrations allowing for correlations between predicted and observed maturity of up to $r=+0.85$ ($P<0.0001$). This procedure will facilitate large –scale wheat phenotyping in the future.

Keywords: climate change; drought; low rainfall; prediction; stay -green;

Abbreviations:: DH, days to heading; DM, days to maturity; Eff, efficiency; GM2, grain number per square meter; GY, grain yield; MaxNDVI, theoretical maximum NDVI; NDVI, normalized difference vegetation index; POP, population; RS, rate of senescence; Stg, NDVI value at physiological maturity; SW, straw weight; TKW, thousand kernel weight; TTMaxNDVI, theoretical thermal time at MaxNDVI;

1. Introduction

One of the most successful applications of physiology in breeding is the analytical exercise of defining traits at the genetic and phenotypic levels that maximize grain yield in different environments based on experimentation and phenotyping (Reynolds and Tuberosa, 2008; Pinto et al., 2010; Reynolds et al., 2011; Cossani and Reynolds, 2012). Trait dissection into maximizing grain yield leverages the ultimate adapted genotype for a specific type of environment, provides a holistic view required for field selection and has been shown to be less susceptible to environmental influences than selecting for grain yield alone (Hammer et al., 2005). Moreover, trait dissection requires new and upgraded methodologies to further automate and successfully apply these traits to better characterize breeding populations. One good example of automation is the use of spectral indexes like the normalized difference vegetation index (NDVI). NDVI has been particularly useful to predict grain yield, vegetation cover and senescence parameters like stay-green when measured at different stages of crop development (Lopes et al., 2014; Raun et al., 2001; Hammer and Schjoerring, 2003; Lopes and Reynolds, 2012). Specifically, stay-green refers to the heritable delayed foliar senescence character in a crop (Thomas and Ougham, 2014).

A stay-green phenotype may be induced by different phenomena: the onset of senescence is delayed, lower senescence rate, lesion of chlorophyll degradation whereas photosynthesis declines or “greenness” is retained due to rapid death at harvest (Thomas and Howarth, 2000). Stay –green in the past has been assessed visually (e.g. Joshi et al. 2007), with SPAD chlorophyll measurements (Harris et al. 2007), with NDVI (Lopes and Reynolds, 2012) and it has been continuously upgraded (Kipp et al., 2013; Christopher et al., 2014) allowing for a more complete view of the different components of stay –green. Previously, a simple model has been used to derive rate of senescence and residual “greenness” at maturity (Lopes and Reynolds, 2012) and this was further expanded to include the onset of senescence, crop or leaf spectral radiance variation after anthesis, and timing from anthesis to on-set (Vilmus et al., 2014). These developments in methodology have mainly contributed to a better understanding of how well the stay –green attributes contribute to grain yield and to grain protein concentration. However, these studies did not address the way these different stay –green components interact to influence final grain yield under low rainfall field conditions with terminal drought in winter wheat. Is there any

compensation between different stay –green components working together for final grain yield? Specific objectives of this study were: 1) to define and estimate senescence parameters and their impact on crop performance in rainfed trials using proximal NDVI spectral radiance measurements in large germplasm collections; 2) to compare senescence parameters in wheat landraces and improved germplasm with basic physiological and agronomic traits; Finally, 3) this study tests NDVI decay curves of different maturing genotypes and tries to identify models to estimate difficult-to-measure traits like days to maturity.

2. Materials and Methods

2.1. Plant materials

Three wheat (*Triticum aestivum* L.) populations were used in this study: population 1 containing a group of 240 landraces from Afghanistan (Kihara collection collected in 1955, by courtesy of Dr. Tomohiro Ban, Kihara Institute for Biological Research, Yokohoma City University) (Manickavelu et al., 2014; Sohail et al. 2015); population 2 containing 250 modern wheat varieties and advanced lines from the International Winter Wheat Program (IWWIP); and population 3 containing 291 landraces from Central and Western Asia (Lopes et al., 2015) from the International Maize and Wheat Improvement Center (CIMMYT) Germplasm Bank.

2.2. Experimental design and environments

Field trials conducted for stay-green modeling were sown during two growth cycles at the Bahri Dagdas International Agricultural Research Institute in Konya, Turkey: in 2012-2013, on October 1st 2012 (populations 1 and 2), and in 2013-2014, on October 24th 2013 (populations 1, 2 and 3); additionally population 2 was grown in 2014-2015 in the same location for predictions of physiological maturity. Konya is located at 37.86472° N and 32.5603° E and 1000 m above sea level, this site has low-to-moderate precipitation and occasional high temperatures during grain-filling (Table 1). As a reference, the two growth seasons used in this study, showed terminal drought typically happening during the last month of grain filling up to maturity and overall both seasons were drier and warmer in the winter (January and February, particularly in 2013-2014)

than long term meteorological data (Table 1). The experimental design was an alpha lattice design with two replicates in plots 1.5 m long with three rows (distance between rows was 25 cm apart and distance between plots was 50 cm) and approximately 200 seeds per m². Soils had 1.1% of organic matter, 2.5 ppm nitrate and a pH of 8.3. Nitrogen fertilization was applied at sowing with 200 kg ha⁻¹ of di-ammonium phosphate and in March with 40 kg ha⁻¹ of ammonium nitrate. Daily weather and soil moisture data were obtained from an IMT 300 (iMETOS) meteorological station and a Watermark Sensor (Irrometer Co., Inc., Riverside, CA) located on site.

2.4. Phenotypic trait evaluation

Grain yield (GY), straw weight and yield components were determined by manually harvesting the middle row of each plot. At harvest, each middle row containing straw and grain was weighed and threshed. The difference between straw plus grain weight and grain weight was used to calculate straw weight. Straw weight was only determined in population 2 and 3 but not in population 1. Thousand-kernel weight (TKW) was determined in samples of 200 grains, and grains per unit area (GM2) were derived from TKW and GY. Days to heading (DH) was determined as the number of days from the sowing date until the point when more than 50% of plants were displaying heads (Zadoks Stage 59, Zadoks et al., 1974). Physiological maturity (DM) was measured as the time when 50% of the spikes in a plot showed a total loss of green color, corresponding to Zadoks stage 89 (Zadoks et al., 1974). Plant height was determined by measuring the distance from the base of the stem to the top of the spike, excluding awns in the field (after physiological maturity) with a ruler. NDVI measurements were taken with a GreenSeeker sensor (Optical Sensor Unit; 2002 Ntech Industries, Ukiah, CA, USA), which records the reflectance in one plot at a frequency of 30-50 times plot⁻¹. NDVI measurements started early March 2013 until the end of June (in the 2012-2013 cycle) and in April 2014 until the end of June (in the 2013-2014 cycle) up to maturity. Measurements continued every one or two weeks up to physiological maturity in 2013 and, in 2014, up to the complete senescence of all plots. NDVI data up to complete senescence was measured in populations 1 and 3 for the 2013-2014 cycle and in population 2 for the 2014-2015 cycle.

2.5. NDVI modeling

2.5.1. Estimating senescence parameters through individual genotype NDVI modeling

NDVI measurements were taken as explained above; in total, nine and ten measurements were taken during the 2013 and 2014 crop seasons, respectively. Daily weather data were used to convert NDVI measurement dates into degree days post heading using base temperature estimated as 5.5 °C as described in Saint-Pierre et al. (2012).

For each plot, we followed a modified procedure described in (Vilmus et al., 2014): specifically, the maximum measured NDVI value through time was identified and all NDVI measurements taken before that date were assumed to correspond to wheat vegetative growth stage. After this date, NDVI decayed and was considered to reflect the wheat senescence stage during grain-filling.

For both vegetative growth and senescence stages, a linear [Eq.1] modeling method was used:

$$[1] \quad NDVI = \beta_0 + \beta_1 t + \varepsilon$$

with $NDVI$ as measured NDVI, t as time in °Cd, β_0 and β_1 as the two parameters of the linear model and ε as the residual term;

Quality of adjustment was determined with Efficiency [Eq.2] to determine how well the two linear models fit to growth and senescence stages,

$$[2] \quad Eff = 1 - \frac{\sum_{i=1}^n (NDVI - \widehat{NDVI})^2}{var(NDVI) \times (n-1)}$$

where $NDVI$ is measured NDVI, $\widehat{NDVI}$ is estimated NDVI and n is the number of data points.

Efficiencies obtained with linear models showed an average of 0.90 across 944 plots with the following distribution: in population 1, the first quartile was between 0.32 and 0.86, the second between 0.86 and 0.93, the third between 0.93 and 0.97 and the fourth between 0.97 and 0.99. In population 2, the first quartile was between 0.55 and 0.90, the second was between 0.90 and 0.95, the third was between 0.95 and 0.97 and the fourth was between 0.97 and 0.99.

Modeling was performed with two linear regressions: one for the growth stage and another one for the senescence stage (Figure 1) using R software v.3.2.0. Linear fitting was calculated with `lm()` function from the `{stat}` package. In our model, NDVI is the explained variable, and time in degree days post heading is the explicative variable, as in Eq. [1].

β_0 and β_1 estimated values were calculated using the least squares method, according to Eq. [3],

$$[3] \quad \min \sum_{i=1}^n (\text{NDVI}_i - \beta_0 - \beta_1 t_i)^2$$

where n is the total number of NDVI measurements over time and NDVI_i is the measured NDVI at time t_i .

Four parameters of interest were further calculated to compare diverse wheat germplasm collections in terms of senescence patterns (Fig. 1): **MaxNDVI**, **TTMaxNDVI**, **RS** and **Stg**; **MaxNDVI** is the theoretical maximum NDVI value over the cycle, calculated as the ordinate of the crossing point between the two regressions corresponding to the vegetative growth and senescence stages; **TTMaxNDVI** is the theoretical thermal time accumulated before (negative value) or after (positive value) heading ('zero' thermal time) corresponding to maximum NDVI, calculated as the abscissa of the crossing point between the two regressions; **RS** is the rate of senescence, calculated as the slope of the senescence regression. **Stg** is the stay-green, calculated as the NDVI value at physiological maturity.

2.5.2. Predicting days to maturity using NDVI

The best model to predict DM was defined by multiple regression using the final rate of senescence (calculated with the last three NDVI measurements at final grain filling stages up to maturity) and DH. The rate of senescence used to estimate days to maturity, unlike RS, was not determined between heading and physiological maturity, instead the last three measurements in time (independently from the growth stage at which the crop was) were used. Theoretically it is expected that early maturing wheat close to the end of the cycle will have a slower rate of senescence than late maturing wheat. Early maturing genotypes have lost almost all their "greenness" at the end of the cycle (lower NDVI loss per unit time) and at the same point in time

a late maturing genotype will have some “greenness” left resulting in higher rates of senescence (higher NDVI loss per unit time). Considering that the previous assumption was correct, physiological maturity would be strongly explained by final rate of senescence (finalRS) and days to heading and this was tested by multiple regression: in population 1, days to maturity was explained by 73% using the rate of senescence and days to heading in multiple regression ($DM = 165.3 + (-170.76 \text{ finalRS}) + (0.368 \text{ DH})$); in population 2, days to maturity was explained by 60% ($DM = 150.498 + (-320.675 \text{ finalRS}) + (0.26020 \text{ DH})$) and in population 3, by 63% ($DM = 186.418 + (-141.76 \text{ finalRS}) + (0.4210 \text{ DH})$). Data used to estimate DM in population 1 and 3 was collected in 2013-2014 and in population 2 was collected in 2014-2015 (where NDVI data was available up to stage at which all plots were completely senesced).

Specifically, to determine the predictive ability of NDVI with final rate of senescence and days to heading to estimate DM, each field trial was divided in two populations: a training population of 20% of all plots and a testing population of the remaining 80% of all plots. The training population was used to define the regression equation of the model using the predictive values obtained from final rate of senescence, days to heading and observed days to maturity (obtained as explained above in this Materials and Methods section, ‘Phenotypic trait evaluation’ subsection). A set of 50 training and testing populations were assembled randomly from each field trial and used to compare predicted and observed values for each model. Finally, estimated and observed values of days to maturity were calculated for each genotype in each trial and plotted.

2.6. Statistical analysis

An analysis of variance was conducted for each year and combined years using an alpha lattice design where genotype was considered as a fixed effect and the effect of block nested with replicate was considered as a random effect with the PROC GLM in SAS (SAS, 2004). Broad-sense heritability (H^2) was estimated for each trait individually to determine their reproducibility across years and within each field trial (by removing the year variance component) as: $H^2 = Vg / [Vg + (Vge/e) + (Ve/re)]$, where r=number of repetitions, e=number of years, Ve=error

variance, V_g =genotypic variance, and V_{ge} =genotype by environment interaction variance. Pearson correlation coefficients on the estimated means and associated probabilities were determined with the PROC CORR from SAS (SAS, 2004) and correlations interpretation was used to deduce some potential physiological mechanisms. Multiple regression was calculated in Excel using the Pop Tools add-in.

3. Results

3.1. Variability for stay-green senescence parameters and agronomic traits in two wheat populations grown for two years

Combined analysis of 2 years for population 1, 2 and 3 are shown in Table 2 and further detailed year by year analysis is shown in Supplementary Table 1 for both populations and years. From the two growth cycles tested, 2013-2014 was the driest one with the lowest grain yields (Table 1 and Supplementary Table 1). In the combined analysis, genotypic effects were significant for all traits across years in population 1 and 2 at $p < 0.05$, except straw weight, showing no significant effects in population 2 (Table 2). In population 3 all traits showed significant genotype effects for one year at $p < 0.05$, except straw weight and RS, showing significance at $p < 0.10$ (Table 2).

For the two first wheat populations, similar correlation patterns between agronomic traits were observed (Table 3). For example, grain yield was highly related to GM2 ($0.8 < r < 0.9$). In population 1 and 2 significant correlations have been obtained between grain yield and the number of days to heading ($-0.2 < r < -0.3$) and TKW ($0.1 < r < 0.3$) (Table 3). In all three populations significant correlations have been obtained between grain yield and PH ($0.2 < r < 0.3$) (Table 3).

Among senescence parameters, RS was negatively related to GY ($-0.1 < r < -0.3$) whereas positive relationships were observed for GY with all the others senescence parameters including MaxNDVI and TTMaxNDVI ($0.1 < r < 0.3$) (Table 3). Similar trends with weaker correlations have been obtained for population 3 (Table 3). Within population 3, traits that best associated with GY and showed positive Pearson correlation coefficients included straw weight ($r=0.6$) and PH ($r=0.2$, Table 3).

To determine the within trial reproducibility of all senescence parameters (without year to year effects), including RS, Stg, TTMaxNDVI and MaxNDVI, heritability, significance of genotypic effects and correlation with GY were determined on a trial basis in each population and year (Table 4). RS was negatively correlated with GY, whereas Stg and MaxNDVI were all positively correlated with GY in population 1 and 2 in both years (Table 4).

3.2. Mechanisms leading to different patterns of senescence in three wheat populations using correlation analysis

In populations 1 and 3 MaxNDVI was correlated with plant height ($0.3 < r < 0.4$), heading or maturity dates ($0.2 < r < 0.3$) whereas there was no significant correlation between these traits in the population containing modern varieties and breeding lines (Table 3). Straw weight which was measured only in populations 2 and 3 was positively related with MaxNDVI ($0.3 < r < 0.4$) (Table 3). Moreover MaxNDVI was significantly associated with RS in the three populations ($-0.4 < r < -0.6$) and with Stg in population 1 and 3 ($0.2 < r < 0.4$, Table 3). TTMaxNDVI was negatively associated with MaxNDVI in populations 1 and 3 ($r = -0.2$) (Table 3). These two parameters (TTMaxNDVI and MaxNDVI) were negatively correlated with RS in all three populations ($-0.2 < r < -0.7$) suggesting the existence of a trade-off between delayed senescence onset and senescence rate or a strong constraint on the date of final senescence. TTMaxNDVI was also negatively correlated with phenology parameters (DH and DM) in all 3 populations ($-0.3 < r < -0.6$, Table 3) and positively correlated with TKW ($0.1 < r < 0.2$) in populations 1 and 2 (Table 3). Lastly, Stg was significantly associated with RS ($r = 0.3$, $r = 0.3$, $r = -0.1$ in population 1, 2 and 3, respectively) (Table 3). Correlations of Stg with DH and DM were significant in population 2 and 3 (Table 3).

3.3. Predicting days to maturity using NDVI decay

NDVI decay was used together with DH to predict DM in populations 1, 2 and 3. This method proved effective for predicting DM when using a calibration or training population containing 20% of all measurements in one trial. Pearson correlation coefficients (r) between observed and predicted DM were on average 0.85, 0.74 and 0.77 in populations 1, 2 and 3 respectively across 50 training and testing sets of populations used for cross-validation (Supplementary Table 2 and

a sample is shown in Table 5). DM was predicted with accuracies of 60-70% (R^2) in all three populations (Fig. 2), when using means of each genotype. Moreover, heritability of predicted DM was on average higher than observed DM in 50 models (Table 5 and Supplementary Table 2).

4. Discussion

4.1. Variability for stay-green senescence parameters in three wheat populations

Worldwide terminal drought is frequent in rainfed winter wheat areas with less than 500 mm of water resulting in considerable yield losses in mega environment 12 (<http://wheatatlas.org/megaenvironments>). An important part of winter wheat area is exposed to less than 500 mm with most of the water falling during the winter and early spring and sharply decreasing afterwards (Lopes et al., 2014). The relevance of stay -green traits has been emphasized in environments with terminal drought (Borrell et al., 2014, Lopes et al., 2012) and herein its contribution to winter wheat grain yield grown under terminal drought has been tested in Turkey. Maximum “greenness” (MaxNDVI), onset of senescence (TTMaxNDVI), rate of senescence (RS) and residual “greenness” at maturity (Stg) were all derived from NDVI growth and decay patterns. These parameters showed important phenotypic variability with significant genotype effects in all three populations. Substantial genetic diversity was observed for senescence traits derived from NDVI growth and decay patterns in wheat **landrace** populations. It was shown that for winter wheat grown under rainfed conditions in Turkey, stay-green is a desirable trait for improving grain yield. This was observed in high yielding stay-green genotypes having: i) higher maximum “greenness” (grain yield was positively correlated with MaxNDVI in population 1 and 2); ii) Delayed onset of senescence (grain yield was positively associated with TTMaxNDVI in populations 1, 2 and 3); iii) increased loss of “greenness” (grain yield was negatively associated with RS in populations 1, 2 and 3); and iv) increased “greenness” at physiological maturity (grain yield was positively associated with Stg in population 1). These results are in accordance with previous reports of significant associations between grain yield and various stay-green traits (Verma et al., 2004; Lopes and Reynolds, 2012; Kipp et al., 2013; Christopher et al., 2014). This methodology seems promising to characterize the stay -green

patterns of large wheat populations exposed to drought and to identify different levels of damage caused by biotic stresses as suggested by Joshi et al. (2007). Moreover, it should be emphasized that NDVI has been showing evidence of saturation with increasing crop stand density (Erdle et al. 2011), however NDVI saturation is not easily attained in the rainfed conditions (low straw weight and yield) of the environments tested in this study.

4.2. Mechanisms leading to different patterns of senescence in three wheat populations

The wheat high yielding phenotype was identified with a delayed onset of senescence (increased TTMaxNDVI), increased maximum “greenness” and straw weight (MaxNDVI), higher rates of senescence (increased RS) and increased residual “greenness” at maturity (Stg) as explained above. MaxNDVI was a measure of total maximum “greenness” and was associated with increased straw weight and PH in populations 2 and 3 where total straw weight was measured. The onset of senescence (TTMaxNDVI) was strongly affected by phenology showing highly significant associations with DH and DM, with delayed onset being observed in early genotypes in all three populations. This could imply that under the water limitations experienced in these experiments, the late genotypes used more water before heading, and this ran out sooner after heading, resulting in earlier onset of senescence. This suggested that the onset of senescence is the stay –green component most affected by the phenological clock of the plant combined with water availability.

Results presented here suggested trade-offs across different stay-green attributes. First, delayed onset of senescence (increased TTMaxNDVI) and increased maximum “greenness” (MaxNDVI) resulted in an increase of RS (absolute values), accelerating the senescence process. In other words, this suggested that a stay–green phenotype with delayed onset of senescence and increased maximum “greenness” during the vegetative stages when water is relatively available will have a pay-off at later stages of grain filling with an accelerated senescence rate when water is scarce in the region (Table 1 shows long term weather data). The rate of leaf senescence can depend on the magnitude of the need for translocation of resources from the leaves (e.g. in sorghum Borrell et al. (2001)). With earlier onset of senescence, more resources may be available from the stem (this would be supported by increased maxNDVI), thus reducing the

requirement for translocation from leaves and finally reducing the rate of leaf senescence (this would be supported by a negative correlation between RS and TTmaxNDVI or onset of senescence). An accelerated senescence rate will decrease total photosynthesis available during grain filling (Thomas and Howarth 2000). This trade –off shows that the most common strategy in these populations to cope with terminal drought is: i) to maximize photosynthesis through increased “greenness” and delayed onset of senescence when water is still available and ii) when water becomes very scarce, senescence is accelerated (high RS) and remobilizes C and N components to the grain with a concomitant increase in grain size (TTMaxNDVI was positively associated with thousand kernel weight). Such tradeoffs have been already observed during grain filling period for N resorption (Vilmus et al., 2014): high resorption rate being negatively correlated ($r=-0.91$) with a delayed onset. In this study trade- offs among senescence components were particularly strong in landrace populations and though present in the population containing modern wheat varieties and breeding lines, these were weaker as shown by the lower Pearson correlation coefficients among stay –green components. These results, suggested that breeders through selection have empirically selected to cope with the trade –offs described above. Ideally, breeders aim at a drought resistant type of wheat, with high “greenness”, delayed onset of senescence and slow rate of senescence, however these genotypes are rare due to compensations or trade-offs among these senescence parameters. In fact, the best way to maximize green leaf area duration during grain filling with terminal drought would be the phenotype showing: highest maximum “greenness”, delayed onset of senescence until close to maturity, followed by rapid rate of senescence. This is not a universal response and the best combination of stay –green parameters to maximize grain yield may vary across different species and environmental conditions: for instance, Harris et al. (2007) showed that slow rates of senescence were favorable to increase grain yield in sorghum. It is concluded, that the use of the rate of senescence to select for stay –green must be coupled with other stay –green components, e.g. onset of senescence and maximum “greenness” to avoid the effects on final grain yield due to trade –offs among stay –green components.

4.3. Predicting days to maturity using NDVI decay

Days to maturity is undoubtedly a very important trait in any breeding program, primarily for farmers who need to decide the maturity types that best suits their rotations. In the past, maturity was measured by observing, plot by plot, when the crop approached maturity and using a few checks of known maturity for comparison (Pask, 2012). Workers would go through each plot at least twice a week until all plots had matured (Pask, 2012). This is time-consuming and comparisons made with other plots in the field are often subjective. NDVI objectively quantifies the loss of “greenness” and has been used with satellite imagery to determine senescence patterns in wheat on a global scale over time (e.g., Lobell et al., 2012). In the present research, wheat maturity was estimated on a plot basis, using the rate of senescence obtained from NDVI decay and heading date. With multiple-regression a training and testing set of 20 and 80%, respectively in each population was used for calibrations allowing for correlations between predicted and observed maturity values of up to $r=0.85$. Moreover, in the future, the same methodology may be applied to remote sensing imagery, making it possible to rapidly and accurately evaluate days to maturity in several thousand of plots.

5. Concluding Remarks

This research will contribute to defining traits for a better adaptation to rainfed winter wheat areas of Central and Western Asia. At this stage, this research is based on a broadly diverse set of germplasm, aimed at balancing major and minor traits that contribute to grain yield in this type of environments. The stay-green phenotype was revealed to be an important trait to improve yields in low rainfall winter wheat growing areas. This study proposes using four stay-green NDVI-derived parameters together, i.e.: onset of senescence, maximum “greenness” attained, rate of senescence and residual “greenness” at maturity. Trade-offs were identified among the different stay-green attributes, e.g. delayed onset of senescence and high maximum “greenness” resulted in accelerated rates of senescence. Moreover, the most successful strategy under terminal drought in Konya, Turkey, included an high maximum “greenness” coupled with a delayed onset of senescence and during grain filling (when terminal drought is experienced), an accelerated rate of senescence accompanied by an increase in grain size. It is concluded, that the

use of the rate of senescence to select for stay –green must be coupled with other stay –green components, e.g. onset of senescence or maximum “greenness” to avoid the effects on final grain yield due to trade –offs among stay –green components.

Using the same NDVI decay curves a final component of senescence was estimated: days to maturity. The correct assessment of days to maturity is undoubtedly a very important trait in any breeding program, primarily to inform farmers about maturity types that best suit their rotations. In this study, NDVI decay during grain-filling and days to heading are used successfully to estimate days to maturity. It is proposed that this advanced method as compared to classical subjective methodologies could be further exploited by high throughput aerial imagery to fully automate the assessment of this trait in breeding programs.

Acknowledgments

Germain Montazeaud was the recipient of the Adanson fellowship funded by the French Agropolis Fondation. Authors would like to thank Dr. Tomohiro Ban, Yokohoma University, Japan, for kindly sharing seeds of Afghan landraces through JST/JICA SATREPS Afghan Wheat Project and the International Winter Wheat Improvement Program (IWWIP based in Turkey) for sharing seeds from the breeding program. This work was funded by the CGIAR Research Program (CRP) WHEAT and the European Union with the project “Addressing the challenges of climate change for sustainable food security in Turkey, Iran and Morocco, through the creation and dissemination of an international database to promote the use of wheat genetic resources and increase genetic gains” approved for funding under the Third Call for Proposals of the Benefit-sharing Fund of the ITPGRFA.

References

Borrell, A.K., van Oosterom E.J., Mullet J.E., George-Jaeggli, B., Jordan, D.R., Klein, P.E., Hammer, G.L., 2014. Stay –green alleles individually enhance grain yield in sorghum under drought by modifying canopy development and water uptake patterns. *New Phytol.* 203, 817-830.

425 Borrell A., Hammer G., van Oosterom E., 2001. Stay-green: A consequence of the balance
 426 between supply and demand for nitrogen during grain filling? *Ann. Appl. Biol.* 138, 91-95.

427 Christopher, J.T., Veyradier, M., Borrell, A.K., Harvey, G., Fletcher, S., Chenu, K., 2014.
 428 Phenotyping novel stay-green traits to capture genetic variation in senescence dynamics. *Funct.*
 429 *Plant Biol.* 41, 1035-1048.

430 Cossani, C.C., Reynolds, M.P., 2012. Physiological traits for improving heat tolerance in wheat,
 431 *Plant Physiol.* 160, 1710-1718.

432 Erdle K, Mistele B, Schmidhalter U (2011) Comparison of active and passive spectral sensors in
 433 discriminating biomass parameters and nitrogen status in wheat cultivars. *Field Crops Res.* 124,
 434 74-84.

435 Hammer, G.L., Chapman, S., van Oosterom, E., Podlich, D.W., 2005. Trait physiology and crop
 436 modeling as a framework to link phenotypic complexity to underlying genetic systems. *Aust. J.*
 437 *Agric. Res.* 56, 947-960.

438 Hammer, P.M., Schjoerring, J.K., 2003. Reflectance measurement of canopy biomass and
 439 nitrogen status in wheat crops using normalized difference vegetation indices and partial least
 440 squares regression. *Remote Sens. Environ.* 86, 542-553.

441 Harris, K., Subudhi, P.K., Borrell, A. Jordan, D., Rosenow, D., Nguyen, H., Klein, P., Klein, R.,
 442 Mullet, J., 2007. Sorghum stay –green QTL individually reduce post-flowering drought-induced
 443 leaf senescence. *J. Exp. Bot.* 58, 327-338.

444 Joshi, A.K., Kumari, M., Singh, V.P., Reddy, C.M., Kumar, S., Rane, J., Chand, R., 2007. Stay
 445 green trait: variation, inheritance and its association with spot blotch resistance in spring wheat
 446 (*Triticum aestivum* L.). *Euphytica* 153, 59-71.

447 Kipp, S., Mistele, B., Schmidhalter, U., 2013. Identification of stay –green and early senescence
 448 phenotypes in high yielding winter wheat, and their relationship to grain yield and grain protein
 449 concentration using high-throughput phenotyping techniques. *Func. Plant Biol.* 41, 227-235.

450 Lobell, D.B., Sibley, A., Ortiz-Monasterio, I., 2012. Extreme heat effects on wheat senescence in
 451 India. *Nature Clim. Change* 2, 186-189.

452 Lopes, M.S., El-Basyoni, I., Baenziger, P.S., Singh, S., Royo, C., Ozbek, K., Aktas, H., Ozer, E.
 453 Ozdemir, F., Manickavelu, A., Ban, T., Vikram, P., 2015. Exploiting genetic diversity from
 454 landraces in wheat breeding for adaptation to climate change. *J. Exp. Bot.* 66, 3581–3598.

455 Lopes, M.S., Reynolds, M., 2012. Stay-green in spring wheat can be determined by spectral
 456 reflectance measurements (normalized difference vegetation index) independently from
 457 phenology. *J. Exp. Bot.* 63, 3789-3798.

458 Lopes, M.S., Saglam, D., Ozdogan, M., Reynolds, M., 2014. Traits associated with winter wheat
459 grain yield in Central and West Asia. *J. Int. Plant Biol.* 56, 673-683.

460 Manickavelu, A., Jighly, A., Ban, T., 2014. Molecular evaluation of orphan Afghan common
461 wheat (*Triticum aestivum* L.) landraces collected by Dr. Kihara using single nucleotide
462 polymorphic markers. *BMC Plant Biol.* 14.320-330.

463 Pask, A.J.D., 2012. Determining key developmental stages. Chapter 14. In: *Physiological*
464 *Breeding II: a field guide to wheat phenotyping*. Pask AJD, Pietragalla J, Mullan DM, Reynolds
465 MP (eds.). Mexico, D.F.: CIMMYT Pp 72-77.

466 Pinto, S.R., Reynolds, M.P., Matthews, K. L., McIntyre, C.L., Olivares-Villegas, J.J., Chapman,
467 S.C., 2010. Heat and drought adaptive QTL in a wheat population designed to minimize
468 confounding agronomic effects. *Theor. Appl. Genet.* 121, 1001-1021.

469 Raun, W.R., Solie, J.B. Johnson, G.V., Stone, M.L., Lukina, E.V., Thomason, W.E., Schepers,
470 J.S., 2001. In-season prediction of potential grain yield in winter wheat using canopy reflectance,
471 *Agron. J.* 93, 131-138.

472 Saint-Pierre, C., Crossa, J.L., Bonnet, D., Yamaguchi-Shinozaki, K., Reynolds, M.P., 2012.
473 Phenotyping transgenic wheat for drought resistance. *J. Exp. Bot.* 63, 1799-1808.

474 Sohail, Q., Manickavelu, A., Ban, T., 2015. Genetic diversity analysis of Afghan wheat
475 landraces using DArT markers. *Genet. Resour. Crop Evol.* 62, 1147-1157.

476 Reynolds, M., Bonnet, D., Chapman, S.C., Furbank, R.T., Manes, Y., Mather, D.E., Parry,
477 M.A.J., 2011. Raising yield potential of wheat. I. Overview of a consortium approach and
478 breeding strategies. *J. Exp. Bot.* 62, 439-452.

479 Reynolds, M., Tuberosa R., 2008. Translational research impacting on crop productivity in
480 drought-prone environments. *Curr. Opin. Plant Biol.* 11, 171-179.

481 SAS Institute Inc., SAS 9.1.3 Help and Documentation, Cary, NC: SAS Institute Inc., 2002-
482 2004.

483 Thomas, H. Howarth, C.J., 2000. Five ways to stay green. *J. Exp. Bot.* 51, 329-337.

484 Thomas, H., Ougham, H., 2014. The stay-green trait. *J. Exp. Bot.* 65, 3889-3900.

485 Verma, V., Foulkes, M.J., Worland, A.J., Sylvester-Bradley, R., Caligari, P.D.S., Snape, J.W.,
486 2004. Mapping quantitative trait loci for flag leaf senescence as a yield determinant in winter
487 wheat under optimal and drought –stressed environments. *Euphytica* 135, 255-263.

488 Vilmus, I., Ecartot, M., Verzelen, N., Roumet, P., 2014. Monitoring N leaf resorption kinetics
489 by near-infrared spectroscopy during grain filling in durum wheat in different N availability
490 conditions. *Crop Sci.* 53, 284–296.

491 Zadoks, J.C., Chang, T.T., Konzak, C.F., 1974. A decimal code for the growth stages of cereals.
492 Weed Res. 14, 415-421.

Table 1-Meteorological data for two wheat growth cycles in Konya, Turkey, in 2012-2013 and 2013-2014. Populations (Pops) grown in each cycle, average minimum temperature (Tmin), average temperature (Tavg), average maximum temperature (Tmax), maximum temperature observed (TmaxObs), Radiation (Rad), precipitation (Prec) and soil water content (SWC) at 0-30 cm, 30-60 cm and 60-90 cm depth are shown. Monthly precipitation and temperature from sowing to harvest are shown as an average of 50 years (Long Term) plus specific years of experimental trials in 2012-2013 and 2013-2014 growth cycles. Pops grown in each year are presented: Population 1 has 240 Afghan landraces, population 2 has 250 breeding lines and varieties and population 3 has 291 landraces from Central and Western Asia.*Available from WorldClimate 1996-2011 (<http://www.worldclimate.com/cgi-bin/grid.pl?gr=N37E032>).

year	Pops	Tmin °C	Tavg °C	Tmax °C	TmaxObs °C	Rad Wm ⁻²	Prec mm	SWC (%)		
								0-30 cm	30-60 cm	60-90 cm
2012-2013	1,2	4.57	11.13	17.42	34.9	158.9	251.6	15.7	15.3	14.6
2013-2014	1,2,3	2.35	9.54	16.34	37.0	165.9	224.9	13.3	13.4	12.2
Sowing								Harvest		
Long Term*	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul
Prec	29.0	31.7	40.4	37.9	30.8	30.0	29.2	45.3	24.5	6.4
Tmin	5.6	0.9	-1.7	-3.7	-2.1	0.4	4.8	8.5	12.5	15.6
Tavg	12.0	6.2	1.7	-0.1	1.6	5.5	11.0	15.7	19.9	23.2
Tmax	19.5	13.1	6.6	4.6	7.4	11.9	17.3	21.9	26.3	29.6
2012-2013										
Prec	34	37.2	63	26.6	33.6	8.6	33	32.6	17	2.2
Tavg	14.2	7.3	3.5	1.5	4.4	6.9	11.3	17.8	21.1	22.7
2013-2014										
Prec.	10.2	6	8.8	58.8	17.4	20.4	19.2	26	31.4	3
Tavg	9.7	7.1	-2.6	2.1	3.9	7.1	12.3	15.4	19.7	25.1

Table 2- Basic statistics for population 1 (240 Afghan landraces), 2 (250 breeding lines and varieties) and 3 (wheat Landraces from Central and Western Asia) grown in Konya, Turkey, for two growth cycles (2012-13 and 2013-14) in population 1 and 2 and 2013-2014 in population 3, with heritability (H^2), Least Significant Difference (LSD), average (AVG), maximum (Max) and minimum (Min), probability values of genotypic effects (p-value G), year effects (YEAR) and genotype by year interaction effects (G*Y).

POP		GY gm ⁻²	GM2 #m ⁻²	TKW g	SW gm ⁻²	DH days	DM days	PH cm	RS Nu°Cd ⁻¹	Stg NDVIu	mN u	TTmN °Cd
1	H^2	0.37	0.54	0.74	NA	0.9	0.8	0.55	0.05	0.29	0.51	0.68
	LSD	77	1694	6.9	NA	4.1	4.5	12.5	0.0003	0.082	0.12	86.4
	AVG	159	3248	34.7	NA	201	235	78.4	-0.0009	0.261	0.7	-31
	MAX	242	6073	49.2	NA	215	242	97.4	-0.0006	0.342	0.83	79.3
	MIN	76	1666	22.2	NA	192	226	57.5	-0.0013	0.152	0.53	-155
	p-val G	<.01	<.01	<.01	NA	<.01	<.01	<.01	<.01	<.05	<.01	<.01
	p-val Y	<.01	<.01	<.01	NA	<.01	<.01	<.01	<.01	<.01	<.01	<.01
	p-val G*Y	0.31	0.83	<.01	NA	<.01	<.05	0.16	<.05	0.14	<.05	<.01
2	H^2	0.13	0.19	0.62	0.26	0.85	0.69	0.58	0.08	0.18	0.13	0.57
	LSD	132	4319	4.9	172	3.4	4.9	12.6	0.00028	0.11	0.12	72
	AVG	236	7645	31.6	434	198	234	67.4	-0.0009	0.24	0.69	-44
	MAX	404	14164	38.8	598	208	2414	94.4	-0.0006	0.36	0.8	63
	MIN	112	2885	24.2	223	193	227	47.5	-0.0014	0.12	0.56	-119
	p-val G	<.05	<.05	<.01	<.10	<.01	<.01	<.01	<.05	<.05	<.05	<.01
	p-val Y	<.01	<.01	<.01	<.01	<.01	<.01	<.01	<.01	<.01	<.01	<.01
	p-val G*Y	<.05	.1	<.01	.25	<.01	<.01	.98	<.05	<.01	<.01	<.05
3	H^2	0.5	NA	NA	0.13	0.9	0.8	0.31	0.15	0.31	0.23	0.15
	LSD	120	NA	NA	270	5.0	3.3	28	0.00072	0.05	0.19	123
	AVG	151	NA	NA	299	205	241	79	-0.0012	0.18	0.71	-104
	MAX	357	NA	NA	839	221	247	118	-0.0007	0.24	0.89	163
	MIN	108	NA	NA	135	191	243	47	-0.0025	0.12	0.49	-236
	p-value G	<.01	NA	NA	<.10	<.01	<.01	<.05	<.10	<.05	<0.01	<.01

Abbreviations: GY, grain yield, GM2, grains per unit area, TKW, thousand kernel weight, DH, days to heading, DM, days to maturity, PH, plant height, RS, rate of senescence, Stg, staygreen or residual NDVI at maturity, mN, maximum NDVI, SW, straw weight, TTmN, thermal time at maximum NDVI. NA, Not applicable; u, NDVI units, °Cd, degree days. NA, not available.

Table 3- Pearson correlation coefficients among physiological and agronomic traits measured in populations 1, 2 and 3 grown in Konya, Turkey. Populations 1 and 2 were grown for two seasons (2012-2013 and 2013-2014) and Pearson correlation coefficients were calculated from overall means of each genotype (estimated means obtained from 2 seasons with N=240 and N=250 for populations 1 and 2, respectively). Population 3 was grown in 2013-2014 and correlations were calculated with N=291 as an average of two replicates. Bold figures indicate significant correlations at $p < 0.05$.

Pop		SW	DH	DM	GM2	PH	RS	Stg	TKW	GY	mN	TTmN
1	DH	NA										
2		0.1										
3		0.3										
1	DM	NA	0.8									
2		0.2	0.8									
3		0.3	0.8									
1	GM2	NA	-0.1	0.0								
2		0.6	-0.2	0.0								
3		NA	NA	NA								
1	PH	NA	0.1	0.3	0.1							
2		0.2	-0.1	0.1	0.2							
3		0.4	-0.1	0.0	NA							
1	RS	NA	0.3	0.3	-0.2	0.0						
2		-0.1	0.0	0.1	-0.1	0.1						
3		-0.3	0.0	-0.1	NA	-0.5						
1	Stg	NA	0.1	0.0	0.2	0.2	0.3					
2		0.0	-0.4	-0.6	0.1	0.0	0.2					
3		0.0	-0.4	-0.5	NA	0.3	-0.1					
1	TKW	NA	-0.3	-0.2	-0.4	0.1	-0.1	0.0				
2		0.0	-0.1	0.0	-0.2	0.2	0.0	0.0				
3		NA	NA	NA	NA	NA	NA	NA				
1	GY	NA	-0.2	0.0	0.8	0.2	-0.3	0.2	0.1			
2		0.6	-0.3	0.0	0.9	0.3	-0.1	0.1	0.3			
3		0.6	0.0	0.1	NA	0.2	-0.2	0.1	NA			
1	mN	NA	0.2	0.3	0.3	0.3	-0.5	0.4	0.0	0.3		
2		0.3	0.1	0.0	0.3	0.0	-0.6	0.1	-0.1	0.2		
3		0.4	0.3	0.2	NA	0.4	-0.4	0.2	NA	0.1		
1	TTmN	NA	-0.6	-0.4	0.2	0.1	-0.3	-0.1	0.2	0.2	-0.2	
2		-0.1	-0.5	-0.4	0.1	0.2	-0.2	0.3	0.1	0.2	-0.1	
3		0.0	-0.5	-0.3	NA	0.3	-0.7	0.2	NA	0.2	-0.2	

Abbreviations: SW, straw weight, DH, days to heading, DM, days to maturity, GM2, grains per unit area, PH, plant height, RS, rate of senescence, Stg, staygreen or residual NDVI at maturity, TKW, thousand kernel weight, GY, grain yield, mN, maximum NDVI, TTmN, thermal time at maximum NDVI, GPC, grain protein content. NA, not available.

Table 4-Basic statistics of stay –green parameters (Heritability, H^2 , correlation with grain yield and associated probabilities, Corr GY(p<)) estimated in individual trials (grown for two seasons in 2012-2013 and 2013-2014 in Konya, Turkey) in two wheat populations (POP 1 and 2).

	2012-2013		2013-2014	
	POP 1	H^2 Corr GY(p<)	H^2 Corr GY(p<)	
RS		0.25 -0.18(<0.01)	0.33 -0.24(<0.0005)	
Stg		0.26 0.22(<0.01)	0.19 0.25(<0.0005)	
TT max NDVI		0.50 0.30(<0.0001)	0.24 0.22(<0.001)	
Max NDVI		0.64 0.38(<0.0001)	0.56 0.24(<0.0005)	
	POP 2			
RS		0.19 -0.12(<0.10)	0.14 -0.34(<0.0001)	
Stg		0.24 0.04(0.54)	0.42 0.23(<0.0005)	
TT max NDVI		0.30 0.13(<0.05)	0.16 0.40(<0.0001)	
Max NDVI		0.21 0.22(<0.0005)	0.52 0.25(<0.0001)	

Abbreviation: RS, rate of senescence, Stg, residual “greenness” at maturity, TTmax NDVI, thermal time at maximum NDVI, MaxNDVI, maximum NDVI.

Table 5- Prediction ability of NDVI to estimate days to maturity (DM) measured in three populations (POP 1, 2 and 3). Random sample of the first 10 models (1-10) tested and actual observations of DM (OBSDM) with all 50 tested models shown in Supplementary Table 4.

POP		Vrep	Vrepxblk	Vent	Vres	H ²	LSD	CORR	O_E	pval
1	OBSDM	0.04	0.06	5.8	2.5	0.83	3.2	NA		NA
		0.00	0.00	5.1	1.1	0.90	2.1		0.85	<.01
		0.00	0.00	5.4	0.9	0.92	1.9		0.85	<.01
		0.00	0.00	3.7	0.6	0.92	1.6		0.85	<.01
		0.00	0.00	4.6	0.8	0.92	1.8		0.85	<.01
		0.00	0.00	3.5	0.7	0.91	1.7		0.85	<.01
		0.00	0.00	3.9	0.7	0.92	1.7		0.85	<.01
		0.00	0.00	4.7	0.9	0.91	1.9		0.85	<.01
		0.00	0.00	4.1	0.7	0.92	1.7		0.85	<.01
		0.00	0.00	4.5	0.7	0.92	1.7		0.85	<.01
		0.00	0.00	5.2	1.0	0.92	2.0		0.85	<.01
		0.00	0.00	5.2	1.0	0.92	2.0		0.85	<.01
2	OBSDM	0.02	0.45	2.9	2.9	0.66	3.4	NA		NA
		0.00	0.15	1.8	1.1	0.77	2.1		0.75	<.01
		0.00	0.15	1.9	1.1	0.77	2.1		0.75	<.01
		0.00	0.24	3	1.8	0.77	2.7		0.75	<.01
		0.00	0.24	3	1.8	0.77	2.7		0.75	<.01
		0.00	0.16	2	1.2	0.77	2.2		0.75	<.01
		0.00	0.1	1.3	0.8	0.77	1.8		0.75	<.01
		0.00	0.1	1.2	0.7	0.77	1.7		0.75	<.01
		0.00	0.2	1.5	1.2	0.73	2.1		0.77	<.01
		0.00	0.16	1.3	1	0.74	2		0.77	<.01
		0.00	0.15	2.3	0.9	0.83	1.9		0.77	<.01
		0.00	0.15	2.3	0.9	0.83	1.9		0.77	<.01
3	OBSDM	0.05	0.06	3.5	2.7	0.72	3.3	NA		NA
		0.05	0.05	2.1	0.7	0.86	1.6		0.77	<.01
		0.04	0.06	2	0.8	0.84	1.7		0.77	<.01
		0.06	0.09	2.9	1.1	0.84	2.1		0.77	<.01
		0.07	0.1	3.3	1.2	0.85	2.2		0.77	<.01
		0.07	0.08	3.1	1	0.86	2		0.77	<.01
		0.07	0.08	3.1	1	0.86	2		0.77	<.01
		0.05	0.06	2.4	0.8	0.86	1.8		0.77	<.01
		0.04	0.04	1.7	0.6	0.86	1.5		0.77	<.01
		0.04	0.06	2	0.7	0.85	1.7		0.77	<.01
		0.04	0.05	2	0.7	0.85	1.6		0.77	<.01
		0.04	0.05	2	0.7	0.85	1.6		0.77	<.01

Abbreviations: variances for Vrep, replicate, Vblock, block, Vrepxblock, replicate by block interaction, Vent, genotype, Vres, residual; H², broad-sense heritability, LSD, least significant difference, CORR O_E, Pearson correlation coefficients for observed DM with Predicted DM, pval, associated probability of CORR O_E.

Fig. 1 NDVI dynamics in two different bread wheat landraces (A & B). NDVI was measured from early March 2013 until the end of June 2013 (2012-2013 cycle) and from April 2014 until the end of June 2014 (2013-2014 cycle) up to maturity and experimental measurements are represented by black points. The first increase corresponds to vegetative growth stage (green trend line) and NDVI decay (red trend line) corresponds to senescence stage. Abscissa axis is thermal time in degree days with $t=0$ corresponding to heading date. Both growth and senescence stages were modeled with linear regressions represented by green and red lines, respectively. Quality of adjustment was calculated with efficiency (Eff, see Eq. 3 in materials and methods). Four parameters were derived from the linear regression estimates: (1) the theoretical maximum NDVI value – max_NDVI, (2) the thermal time at theoretical maximum NDVI – TT_max_NDVI (intersection of growth and NDVI decay highlighted by a blue circle), (3) the rate of senescence – RS and (4) the NDVI value at physiological maturity, or stay-green – Stg.

Fig. 2- Association of predicted and observed days to maturity by multiple regression of final rate of senescence based on NDVI decay during grain-filling and days to heading to predict days to maturity. A, B and C correspond, respectively, to populations 1 (Afghan landraces), 2 (modern lines and varieties) and 3 (landraces from Central and Western Asia). Genotypic means with N=240, N=250 and N=291 for populations 1, 2 and 3, respectively, were used for linear regression. Equations of regression lines, R^2 and associated p values are shown.