The Use of Chlorophyll Meter Readings for the Selection of Maize Inbred Lines under Drought Stress

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Abstract**—**The present study aimed to investigate whether chlorophyll meter readings (SPAD) can be used as criterion of single-plant selection in maize breeding. Experimentation was performed at the ultra-low density of 0.74 plants/m^2 in order the potential yield per plant to be fully expressed. R-31 honeycomb experiments were conducted in three different areas in Greece (Thessaloniki, Giannitsa and Florina) using 30 inbred lines at well-watered and water-stressed conditions during the 2012 growing season. The chlorophyll meter readings had higher rates at dry conditions, except location of Giannitsa where differences were not significant. Genotypes of highest chlorophyll meter readings were consistent across areas, emphasizing on the character's stability. A positive correlation between the chlorophyll meter readings and grain yield was strengthening over time and culminated at the physiological maturity stage. There was a clear sign that the chlorophyll meter readings has the potential to be used for the selection of stress-adaptive genotypes and may permit modern maize to be grown at wider range of environments addressing the climate change scenarios.

Keywords**—**Drought-prone environments, honeycomb breeding, SPAD, *Zea mays*.

I. INTRODUCTION

DROUGHT is one of the major environmental stresses that limit plant growth, productivity and consequently, crop yield [1]. Crop plants respond to water deficit and adapt to drought conditions through various molecular, physiological, and biochemical changes. Some of these changes can be measured and have been proposed for the selection of new genotypes that are tolerant to drought stress; however, their use is limited.

Loss of yield is the main concern of plant breeders and hence they emphasize yield performance under stressed conditions. However, the complexity of grain yield caused by large genotype–season and genotype–location interactions make it difficult to select stress-tolerant genotypes, and variation in yield potential can arise from factors related to adaptation rather than to drought tolerance per se [2]. Thus, it is necessary to measure secondary traits as well as grain yield to evaluate genotypes objectively and improve the precision of selection. In maize, secondary traits related to drought resistance include ears per plant, anthesis–silking interval (ASI), leaf rolling, tassel size, chlorophyll meters and stay green; all can be used for preliminary selection and are also genetically associated with grain yield under drought, as well as being genetically variable and highly heritable [3].

Comparison between old and recent maize hybrids showed that selection for high yield potential is intimately linked to selection for stress resistance; with tolerance to weed interference, low soil nitrogen and low soil moisture among the key factors that sustained the genetic gain in maize [4], [5].

Stay green plants are characterized by a postflowering drought resistance phenotype that gives plants resistance to premature senescence, stalk rot, and lodging when subjected to drought during grain-filling. Stay green has been extensively used in plant breeding to improve yield potential and yield stability in all environments, including drought-prone areas [4], [6]. Strong evidence in support of the value of stay green characteristics in the adaptation to abiotic stresses comes from retrospective comparisons of the performance of temperate maize hybrids produced over the last 70 years in the USA [7].

C4 plants have high water use efficiencies (WUEs), and the presence of the CO_2-concentrating mechanisms makes C4 photosynthesis more competitive in conditions that promote carbon loss through photorespiration, such as high temperatures, high light intensities, and decreased water availability [8]. Responses to drought are species specific and often genotype specific [6].

Maize grain yield increased from about 1,500 kg/ha in the early 1900s to 8,500 kg/ha at the beginning of the 2000s in the USA [9], [10]. Despite this spectacular achievement, maize grain yield is closely related to plant population density [11], and the crop suffers from an agronomic weakness of prime significance, affecting its grain productivity and stability. Modern hybrids are usually population dependent [12], [13], with the ideal plant number per area depending on several factors, including water availability, soil fertility, hybrid maturity group, and row spacing [14].

Reference [15] shows that there have been defined three categories of competition where a given genotype may be evaluated. These categories are the 'isolation environment', the 'crop environment' and the 'competition environment'. In

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the isolation environment, individual plants are spaced so widely apart as to eliminate any plant-to-plant interference for the equal use of growth resources [15]. Because individual plants are not affected by the competitive ability of neighboring plants, the condition is deemed as ‘nil-competition’. So every plant in the stand is reliant solely on its own genetic potential throughout the whole developmental cycle, from emerge to the reproductive stage. Therefore, the isolation environment assesses accurately the full genetic potential of single plants for all the traits measured [15]-[17].

The objective of the present study was to investigate whether chlorophyll meter readings (SPAD) can be used as criterion of single-plant selection in maize breeding.

II. MATERIALS AND METHOD

A. Experimental Setup and Measurements

Experimentation was performed at the ultra-low density of 0.74 plants/m^2 in order the potential yield per plant to be fully expressed (Fig. 1). This density corresponding to a plant-to-plant spacing of 1.25m and the thirty inbred lines and one extra inbred line as a control were grown in a honeycomb-31 replicated trial [18]. If d = 1.25m is the plant spacing used in the honeycomb design, this corresponds to 0.74 plants/m^2 [18]. The low density was used to assess the yield potential and homeostasis of the hybrids according to the second of the two developed equations [19]. R-31 honeycomb experiments were conducted in three different areas in Greece. The first location (Site 1) was in Florina region (40° 46' 41.50", 21° 22' 46.09" a loam-sandy loam soil with pH 6.3 and organic matter 14.0g/kg). The second location (Site 2) was at the Aristotle University Farm of Thessaloniki (40°32′N, 22°59′E, 6m asl, a clay loam soil with pH 7.6, organic matter 25.8g/kg, N-NO$_3$ 17.9mg/kg, P-Olsen 25mg/kg and K 109mg/kg). The third location (Site 3) was Giannitsa (40° 45' 58.64", 22° 24' 18.57"). The soil characteristics were determined according to methods detailed in [20]. The preceding crop was winter wheat (*Triticum aestivum* L.).

Source material consisted of 30 inbred lines and evaluated at well-watered and water-stressed conditions during the 2012 growing season (Fig. 2). The planting dates were 27 April for the trials grown in Thessaloniki, 4 May in Giannitsa and 6 May in Florina. Nitrogen and P fertilizers were applied at the rate of 120 and 60kg/ha respectively, in all three areas. The fertilizers were incorporated with a tandem harrow disc to a depth of 12-15cm after application.

Chlorophyll content was measured at four developmental stages: at anthesis and 14, 28 and 42 d post-anthesis, comprising respectively SPAD1 to SPAD4 using the SPAD-502 chlorophyll meter (Minolta Co Ltd, Osaka, Japan). The portable instrument determines the amount of chlorophyll present based on the absorbance of two wavelengths of light (650 and 940nm) passing through intact leaves. Chlorophyll content was measured in all plants having no competing neighboring plants. Measurements were taken on ear leaf of the topmost ear. Three measurements were taken, near the base, middle, and midway between the midrib and the leaf margin [21]. The average of the 3 readings was recorded for each individual plant.

For the dry grain weight individual plants of the honeycomb trials were harvested and the yield per plant was measured.

Fig. 1 Honeycomb arrangement, where the entries are laid out in an equilateral triangular lattice (ETL) pattern that ensures comparable allocation of entries to environmental diversity

Fig. 2 Inbred lines of maize grown in the field at the Aristotle University Farm of Thessaloniki

B. Experimental Design and Statistical Analysis

The experimental was arranged in a split-split-plot design with the three locations as the main plot factor, the irrigation treatments as the sub-plot factor and the different inbred lines as the sub-sub-plot factor. Combined analysis of variance (ANOVA) was performed across locations (L) for yield (DGW) and physiological traits for individual measurements (SPAD1-3). The treatment sum of squares (SS_{TRMT}) was partitioned into inbred lines (SS_G), irrigation treatment (SS_W), and the two and three way components (SS_{LW}, SS_{GW}, SS_{GL}, SS_{GWL}) as percent (%) sums of squares [22]. Pearson correlation coefficients were also calculated for both physiological and yield traits [23]. All statistical analyses were performed using the SPSS ver. 17 software package (SPSS Inc., USA, IL: Chicago).

The monthly temperature (^{o}C) and precipitation (mm) means and the relative humidity for the locations of Florina and Thessaloniki are given in Figs. 3-5. In Florina, the spring was quite mild and there was more rainfall during the summer. In contrast, in Thessaloniki was warm during the spring and there was less rainfall during the summer (Figs. 3-5).

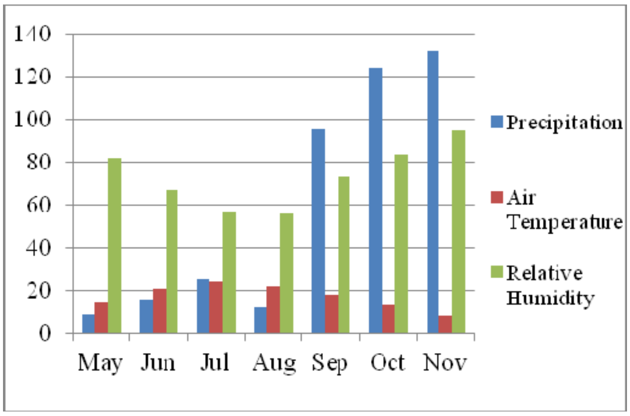

Fig. 3 Monthly precipitation (in mm), average of air temperature (C^o) and relative humidity (%) for the area of Florina for the 2012 growing season

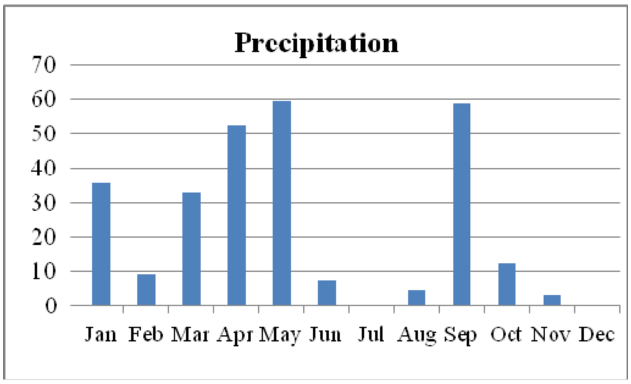

Fig. 4 Monthly precipitation in mm for the Aristotle University Farm of Thessaloniki, for 2012 growing season

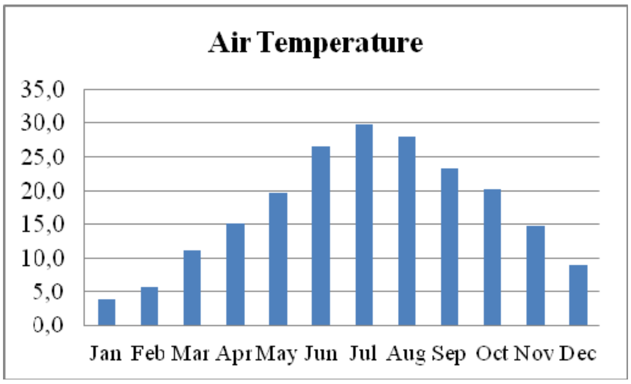

Fig. 5 Monthly average of air temperature (^oC) for the Aristotle University Farm of Thessaloniki, for 2012 growing season

III. RESULTS AND DISCUSSION

SPAD readings were significantly affected by genotypes and locations and their interactions (with the exception of SPAD2 for L). As Table I shows, in all measurements, SPAD was highly affected by genotypes (G). A high contribution (66.4, 76.8 and 59.9%) of G effect on SS_{TRMT} was found for SPAD1-3 respectively. On the contrary, the factor of the irrigation treatment and all its interactions had no effect to all SPAD measurements. All SPAD measurements had the same behavior with the factors and the interactions that affected them.

TABLE I
ANALYSIS OF VARIANCE AS PERCENT (%) CONTRIBUTION TO TREATMENT SUM OF SQUARES (SS_{TRMT}) FOR EACH OF THE THREE MEASUREMENTS (SPAD1-SPAD3) CONDUCTED FOR THE 30 MAIZE INBRED LINES TESTED.

Source of variance	df	SPAD1	SPAD2	SPAD3
Locations (L)	2	9.6*	0.1	17.0*
Water (W)	1	0.1	0.4	0.0
L×W	2	0.1	0.0	0.2
Genotypes (G)	29	66.4*	76.8*	59.9*
G×W	29	5.6	6.8	3.1
G×L	58	14.1*	10.6*	14.6*
G×W×L	58	4.1	5.3	5.2

*Significant at the 0.05 probability level.

The means over the growing season for the physiological trait (MSPAD) along with the individual measurements with the SPAD1 were used to correlate with yield. The individual measurement SPAD1 was strongly correlated with the respective mean MSPAD meaning that there was good indicator of the inbred lines response during the course of the growing season (Table II). SPAD measurements (MSPAD and SPAD1-4) gave positive and significant correlations with yield (Dry Grain Weight). As well, a positive correlation between the chlorophyll meter readings and grain yield was strengthening over time and culminated at the physiological maturity stage. On the contrary, MSPAD did not correlate significantly with Harvest Index, but the individual measurements show significant correlations except SPAD4, which did not correlated significantly (Table II).

Chlorophyll meters readings have been used in many different crop species and especially in many annual crops, as they provide a valuable estimation of the N status [24]-[28]. However, their use in plant breeding is limited and especially for the selection under abiotic stress, such as under drought stress.

Other researchers found differences for chlorophyll concentration among maize inbreds and hybrids but no observed correlation between chlorophyll and grain yield [29]. However, path coefficient analysis suggested that chlorophyll concentration had a large positive correlation with grain yield via elongation of grain-filling period and kernel number [30].

TABLE II
CORRELATIONS BETWEEN PHYSIOLOGICAL TRAIT (MSPAD, SPAD1-SPAD4) WITH YIELD (GRAIN DRY WEIGHT-GDW) FOR THE 30 MAIZE INBRED LINES TESTED ACROSS TWO TREATMENTS AND THREE LOCATIONS

	MSPAD	SPAD1	SPAD2	SPAD3	SPAD4
SPAD1	0.43*				
GDW	0.27*	0.25*	0.27*	0.26*	0.33*
HI	0.06	0.13*	0.23*	0.18*	0.04

*Significant at the 0.01 probability level.

The chlorophyll meter readings had higher rates at dry conditions, except Site 3 where differences were not significant (Fig. 6). Genotypes of highest chlorophyll meter readings were consistent across areas, emphasizing on the character's stability. Selection for chlorophyll content could potentially be an effective physiological trait worth using in breeding programs aimed at improving photosynthetic capacity and dry matter accumulation [31].

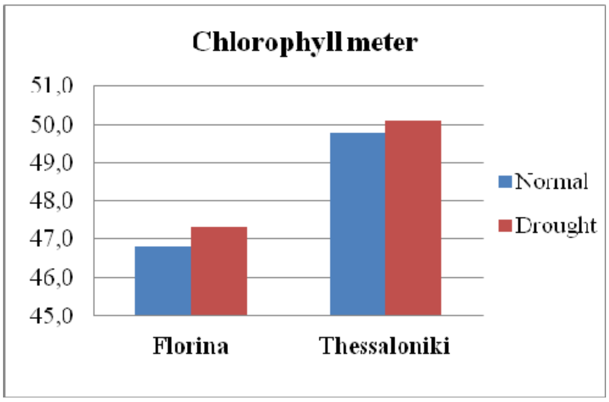

Fig. 6 Chlorophyll meter readings (SPAD) at two locations (Florina and Thessaloniki) and two treatments, foul water (normal) and 60% water (drought)

SPAD readings have been proven an easy, non-destructive and reliable assessment of leaf greenness via their association with chlorophyll content and consequently with leaf N concentration in many species, maize included [32]. Thus, SPAD readings can be a handy monitor of leaf photosynthetic machinery, able to capture in season changes caused by environmental factors. To serve this role, SPAD should not be biased by factors like leaf ontogeny, which dissipate its association with chlorophyll and N [33], [34].

IV. CONCLUSION

The results of this study suggest that chlorophyll meter readings could be used in the selection of maize inbred lines tolerant to water stress.

There was a clear sign that the chlorophyll meter readings has the potential to be used for the selection of stress-adaptive genotypes and may permit modern maize to be grown at wider range of environments addressing the climate change scenarios.

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