Genetic Analysis of Staygreen Trait and its Association with Morpho-physiological Traits under Water Deficit Stress in Wheat

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The present study was conducted to assess the relation of staygreen trait with yield attributes and physiological traits under water deficit condition in wheat and to identify genetically diverse and agronomically desirable functional staygreen parents for incorporation in the breeding programmes for the development of drought tolerant genotypes. Heritability estimates along with genetic advance are high for leaf senescence rate (LSR). Present study also demonstrated significant positive association of staygreen trait LSR with RWC, photosynthetic rate, ear weight, thousand kernel weight and grain yield. Grouping genotypes for drought tolerance and staygreen trait suggested, CHIRYA7 as the most drought tolerant (DSI = 0.58) staygreen genotype (LSR=0.13) and CBW38 as drought susceptible (DSI = 1.12) fast senescing (0.48) genotype. D²-clustering grouped the genotypes into six clusters on the basis of similarity in the traits studied. Cluster III genotypes display staygreen character because it exhibited maximum cluster mean for photosynthetic rate, grain yield, test weight, SCMR, ear weight, biological yield, RWC and minimum cluster mean for DSI and LSR. There is a maximum intercluster distance between Cluster III and cluster VI hence staygreen genotypes from cluster III viz. CHIRYA7, HW2041 can be selected and crossed with cluster VI genotypes viz. HW2063 for incorporation of functional staygreen trait for development of drought tolerant genotypes. The clustering of genotypes will help to identify divergent genetic material for obtaining highly heterotic functional staygreen wheat hybrid for drought condition.

Key Words: D² clustering, Leaf senescence, Staygreen trait, DSI, Wheat, Drought

Introduction

Wheat (T. aestivum L. em Thell.) is a staple food for more than 35% of the world population and covers 30% of the world's cereal producing area. Abiotic stress affects 220 million hectare of wheat producing area worldwide which consequently lowers its yield (Cossani et al., 2012). Wheat yields have been reported to reduce by 50-90% of their irrigated potential by drought (Reynolds et al., 2005) Thus, breeding for drought tolerance is a major objective in crop improvement programme. Traits associated with post flowering drought tolerance include improved rooting depth (Sharp et al., 2004), stay-green (Rajcan and Tollenaar, 1999; Borrell et al., 2000), longer grain filling duration, increased grain filling rate and increased individual grain weight (Harris et al., 2007). Drought leads to premature leaf senescence which decreases leaf area duration consequently resulting in yield reduction. Senescence rate in wheat is particularly sensitive to water and heat stress and like many other traits, genetic variation for this trait has been reported (Falqueto et al., 2009; Srivalli and Chopra, 2009).

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Genotypes possessing the ability to maintain green leaf area duration throughout grain filling are termed as "staygreen" (SG) and are potential candidates to assure yield in semi-arid regions. SG genotypes have been used successfully in sorghum for yield stability and promises as a selection tool in wheat (Christopher et al., 2008). Therefore, selection of slow senescing genotypes with high yield stability under drought condition should be the selection criterion for functional staygreen genotypes. Modern cultivars in wheat and other crops are often genetically similar, with a rather narrow genetic base. The presence of genetic diversity is important for successful wheat breeding because artificial crossing among dissimilar parents allows segregation and recombination of different favourable alleles (Bered et al., 2002). A wide array of material including wheat land races, local varieties, advanced lines, and crosses with ancestral species are being used by different wheat breeding programmes in the country to generate the necessary genetic diversity. Better understanding of the genetic basis of this variability will improve the efficiency of wheat improvement for drought tolerance. The present study was undertaken to identify genetically diverse and agronomically desirable functional staygreen parents for developing high yielding functional staygreen wheat genotypes under drought condition.

Materials and Methods

During Rabi (2012-2013) a pot culture experiment was conducted on thirty five core elite wheat germplasm from India and CIMMYT, Mexico. Sowing was done in earthen pots (about 30 cm in diameter and 30 cm in depth) with four plants per pot filled with clay loam soil and farmyard manure (FYM) in 3:1 ratio during rabi (winter) season. Nitrogen, phosphorus and potash fertilizers were applied at the rate of 60: 60 kg per hectare, respectively in the form of urea, single super phosphate and muriate of potash at the time of sowing. Remaining 60 kg N ha⁻¹ was given after 25 days of sowing. Plants were subjected to water deficit stress for eight days after anthesis (DAA) by withholding irrigation (RWC 65-70%), while in irrigated plants RWC ranged from 80-85%. The response of plants in terms of growth and physiological traits were studied in upper most fully expanded flag leaf.

Photosynthetic Rate

Leaves were categorized into green and yellow/dead, and the rate of photosynthesis was measured using portable Infrared Gas Analyser (IRGA), Model LI-6400XT (Li-COR Ltd., Lincoln, Nebraska, USA) by operating it in the closed mode between 10.00-11.00 a.m. when relative humidity, temperature, photosynthetic photon flux density and CO₂ concentration ranged from 50-60%, 30 to 35°C, 1200 µmol m⁻²s⁻¹ and 350 to 360 µmol mol⁻¹, respectively. Fifteen flag leaves per treatment were selected at random for photosynthetic rate measurement and expressed in µmol CO₂ m⁻² s¹.

SPAD Chlorophyll Meter Reading (SCMR)

Soil and plant analyser development (SPAD) values were measured in the middle part of flag leaves using portable Minolta SPAD-502 chlorophyll meter (Minolta camera Co. Ltd., Osaka, Japan) after flowering at the end of stress period for eight days. The average reading of 10 leaves per pot was recorded and used in the analysis. Measurements were carried out twelve times between flowering and the end of senescence on three flag leaves for each genotype.

Leaf Senescence Rate

Phenotyping for LSR was done visually and senescence score was estimated, dividing the percentage of estimate area that is dead by time duration in days (Lu *et al.*, 2011). The twelve dates of assessments were expressed as $(\Sigma t_1 - \Sigma t_{12})$ and the corresponding senescence score $(S_1 - S_{12})$. The average senescence (S_a) was calculated after 20 days from 50% anthesis as $(S_i+_{20}-S_i) / (\Sigma t_i+_{20} - \Sigma t_i)$ as given by (Guendouz et *al.*, 2012).

Yield and Yield Components

The plants were harvested separately from control and water stressed pots. Measurements on grain yield per plant were recorded as economic yield. The whole plant dry weight was measured as biological yield. Three replications were taken for each parameter.

Drought Susceptibility Index (DSI)

The drought susceptibility index was calculated by using the formulae of Fisher and Maurer, 1978.

$$S = (1 - Y/Yp)/D$$

Where S is drought susceptibility index, D is stress intensity, Y is yield under stress, Yp is yield without stress and

D = Stress intensity = 1-X/Xp

Where X and Xp represent average varieties yield under stress and non-stress conditions respectively. The S was used to characterize the relative drought stress tolerance of the various species (S \leq 0.50 high drought tolerant, S \geq 0.50 \leq 1.00 moderately stress tolerant and S>1.00 Susceptible).

Statistics

The data obtained from the experiments was subjected to analysis of variance (ANOVA) by complete randomized design and F-test was carried out to test the significance of the treatment differences. The correlation was done using MS Office Excel. The genetic divergence was estimated by using D^2 statistics of Mahalanobis, 1936, and the grouping of the genotypes into clusters was done using Euclidean method of clustering. The INDOSTAT software version 9.2 was utilized for statistical analysis.

Result and Discussion

The analysis of variance was highly significant among the divergent genotypes for all the morpho-physiological traits under study, which revealed the presence of considerable variability among the studied genotypes.

Genetic Analysis

Genotypic coefficient of variation, phenotypic coefficient of variation, heritability (broad sense), genetic advance and genetic advance expressed as percent of mean for 12 characters are shown in Table 1. The PCV values were higher than GCV values for all the traits that reflects the influence of environment on traits. The estimates of GCV and PCV were high for all the characters studied except SCMR value, relative water content and plant height which showed the moderate GCV and PCV (Table 1). High heritability coupled with high genetic gain for LSR indicates that it exhibits additive gene effect and is not influenced by environmental effects which is in agreement with Chen et al., (2013) who showed that the broad-sense heritabilities of staygreen trait such as ratio of visible green leaves at physiological maturity in maize were relatively high. It indicates that selection may be effective in early segregating generation for these traits under water deficit. Therefore, there seems a scope for improvement in these traits or selection would be effective in water deficit condition.

Genetic Correlation Studies

LSR shows significant negative correlation with SCMR (-0.59) indicating that drought induced leaf senescence leads to reduction in chlorophyll (Table 2). Likewise, Guendouz *et al.*, (2012) also reported significant negative correlation between chlorophyll content and average senescence (r = -0.68). Chlorophyll is the

Table 1. Estimates of genetic parameters for 12 quantitative characters of 35 wheat genotypes.

Characters	GCV	PCV	h ² (bs)	GA	GG
LSR	33.020	34.320	93.069	0.205	65.624
SCMR	6.321	12.150	27.036	0.300	6.764
Pn rate	60.264	62.061	94.261	8.596	90.514
RWC	7.660	9.771	61.469	6.611	12.377
PHT (cm)	10.481	14.361	53.302	12.664	15.763
TPP	32.400	34.680	87.277	5.209	62.359
EWP (g)	47.021	49.371	90.672	7.544	92.223
TKW (g)	33.472	36.040	86.269	19.223	64.047
BYP (g)	32.871	36.541	80.957	11.561	60.933
HI (%)	33.961	36.320	87.456	16.157	65.431
GYP (g)	50.291	51.923	93.840	4.746	100.361
DSI	20.262	20.371	98.943	0.417	41.508

 $GCV = Genotypic \ coefficient \ of variation, PCV = Phenotypic \ coefficient \ of variation, h² = heritability, GA = Genetic Advance & GG = Genetic gain$

major photosynthetic pigment in plants which functions to capture and transfer light energy. A functional staygreen is defined as retaining both leaf greenness and photosynthetic competence much longer during leaf senescence, while non-functional staygreen is defined as maintaining only leaf greenness (Thomas and Smart, 1993; Thomas and Howarth, 2000). LSR shows positive association with the photosynthetic rate established by the fact that it shows significant negative correlation with the photosynthetic rate which is in agreement with Patro et al., (2014) who showed that during dehydration induced leaf senescence in Arabidopsis thaliana leads to reduction in photosynthesic rate due to decrease in primary photochemical reaction of thylakoids. SG trait also show significant correlation and positive association with yield determining components such as ear weight per plant, 1000 kernel weight, biological yield and harvest index under drought (Table 2). This positive association of SG trait with other yield attributes consequently lead to its significant correlation (LSR= -0.49) with grain yield under drought condition. These positive association of staygreen trait with ear weight and grain yield is positive which is in agreement with Distelfeld et al., (2014) who illustrated that wholeplant senescence overlaps with grain filling, and the synchronization of these two processes is highly important in determining yield, particularly through the grain weight component. It is evident from this positive association of staygreen trait with test weight which was supported by the observations of Spano et al., (2003) that 'staygreen' mutants of durum wheat maintained photosynthetic competence for longer time than the parental line, attained a higher grain weight. LSR show significant significant negative correlation with drought susceptibility index (DSI) which is in agreement with Jordan et al., (2012) who showed that staygreen trait is positively correlated with sorghum grain yield in field conditions under terminal drought.

Classification of genotypes on the basis of average leaf senescence rate and drought susceptibility index

Genotypes for staygreen trait were selected on the basis of LSR and (DSI). The mean value of LSR and DSI. The mean value of DSI and LSR was 0.31 and 1.00 respectively. There are ten genotypes with less than average LSR and DSI and these were identified as functional staygreen genotypes (Fig. 1), 13 genotypes with more than average LSR and DSI were identified

Character	LSR	SCMR	Pn rate	RWC	PHt	TPP	EWP	TKW	BY	HI	DSI
LSR	1.0										
SCMR	-0.59***	1.0									
PR	-0.33*	0.87****	1.00								
RWC	-0.97****	0.69****	0.48***	1.0							
PH	0.18*	0.13 ^{ns}	0.07 ^{ns}	-0.09 ^{ns}	1.00						
TPP	0.06 ^{ns}	0.26 ^{ns}	0.24*	0.04 ^{ns}	0.33	1.00					
EWP	-0.61****	0.73****	0.40***	0.80****	0.05 ^{ns}	0.39*	1.00				
TW	-0.52***	0.59***	0.61****	0.65****	-0.27*	-0.10 ^{ns}	0.45**	1.00			
BY	-0.35*	0.73****	0.43***	0.42***	0.32*	0.66****	0.71****	0.13 ^{ns}	1.00		
HI	-0.34*	0.15 ^{ns}	0.01 ^{ns}	0.37**	0.17*	-0.28 ^{ns}	0.25 ^{ns}	0.38*	0.02 ^{ns}	1.00	
DSI	0.54***	-0.61****	-0.21 ^{ns}	-0.66****	-0.37**	-0.14 ^{ns}	-0.67****	-0.45**	-0.53***	-0.63****	1.00
GY	-0.50**	0.66****	0.37*	0.64****	0.31**	0.16ns	0.67****	0.51***	0.55***	0.65****	-0.85****

Table 2. Correlation of staygreen trait with other drought tolerance and yield attributes in wheat under drought condition

**** means significance at 0.01% level of significance, *** means significance at 0.1% level of significance, ** means significant at 1% level of significance, * means significant at 5% level of significance, ns means non significant.

LSR= Leaf senescence rate, GI=Grenness index, Pn rate=Photosynthetic rate, RWC=Relative Water content, PHt=Plant height, TPP= tillers per plant, EWP=Ear weight per plant, TKW=1000 kernel weight, BYP=Biological yield per plant, HI=Harvest index, GYP= grain yield per plant, DSI= Drought susceptibility index.



Fig. 1. Genotypes Classified on the basis of average LRS watered and DSI under water deficit condition. (A) identifies functional staygreen genotypes exhibiting low LSR and low DSI. (B) identifies non-functional staygreen genotypes (Cosmetic staygreen genotypes) exhibiting low LSR and high DSI. (C) identifies non-staygreen genotypes exhibiting high LSR and high DSI. (D) identifies genotypes exhibiting High LSR and Low DSI (functional non-staygreen).

as non-staygreen genotypes, eight genotypes with low LSR and high DSI were identified as cosmetic staygreen genotypes, and four genotypes with high LSR and low DSI were identified. Out of ten functional SG genotypes, CHIRYA7 and HW2041 have been selected as agronomically desirable functional staygreen parents with maximum SCMR, photosynthetic rate, grain yield and grain weight. This is in agreement with (Christopher *et al.*, 2008; Gous *et al.*, 2013) who shows that staygreen mutants in various wheat cultivars have increased grain weight and yield as a result of delayed senescence and also perform better under water deficit stress.

Genetic Diversity Analysis

On the basis of Mahalanobis D^2 following Euclidean method for clustering, thirty five genotypes were grouped

Cluster group	No. of genotypes	Name of genotypes
Cluster I	6	PBW373, HW2060, HW2055, HW2008, HW 2085, HD2894
Cluster II	4	HW2061, HW2027, HW4010, HW2042
Cluster III	4	CHIRYA7, HW2041, HW4024, PBW502
Cluster IV	9	PBW555, CHIRYA3, CHIRYA1, LOK-64, HW2020, HW4050, GW-322, HW4030, HW4009
Cluster V	11	CBW-38, HW2033, UP-2696, HW4060, WL711, HW2080, HW2051, HW4007, Shanghai1, HW4203, HD2789
Cluster VI	1	HW 2063

Table 3. Distributing pattern of 35 genotypes of wheat into six clusters based on Euclidean analysis

Table 4. Average intra and inter cluster D² values for 35 genotypes of wheat

	1 Cluster	2 Cluster	3 Cluster	4 Cluster	5 Cluster	6 Cluster
Cluster I Cluster II	118.918	283.136 167.568	306.899 344.647	385.231 280.79	601.232 318.263	1133.336 708.719
Cluster III			150.869	731.943	886.635	1382.667
Cluster IV				96.694	157.141	373.980
Cluster V					93.086	280.959
Cluster VI						0.000

into six clusters with cluster-wise variable number of genotypes (Table 4 Fig. 2) developed by various centres and located at different geographical locations. A distribution pattern of all the genotypes into various clusters (Fig. 2) showed the presence of considerable genetic divergence among the genotypes for most of the studied traits. Amongst six clusters, cluster VI was mono-genotype cluster, whereas, cluster V was the largest having 11 genotypes involving varieties/ strains from various centres. Similarly, cluster II and III each consisted of four genotypes and cluster I and IV consisted of nine genotypes. Amasiddha et al., (2013) also investigated genetic diversity for moisture deficit stress adaptive traits in bread wheat and classified the 33 genotypes of into six clusters. It was observed that intra cluster distance was maximum for cluster II (167.56) followed by cluster III (150.86) and cluster I (118.91), while cluster IV (96.69) and cluster V (93.08) had slightly lower values of intra-cluster distance. Thus, genotypes included within the cluster I, II and III revealed maximum diversity. The highest inter-cluster distance was noted between cluster III and cluster VI which is followed by cluster I and VI (1133.33), III and V (886.63) and cluster III and IV (731.94). Maximum cluster mean for the character grain yield per plant was observed for the cluster III (8.677). Cluster III, was selected for higher value of SCMR, photosynthetic rate, RWC, ear weight per plant, test weight, harvest index and grain yield. Cluster V has lowest RWC, Ear weight and test weight and low biological yield, grain

yield, harvest index and categorized as fast senescing and drought susceptible due to maximum LSR (0.421) and DSI (1.183). Hence, cluster III genotypes can be categorized as functional staygreen exhibiting minimum cluster mean. for LSR and DSI. All the 10 genotypes out of 11 present in cluster V overlapped with the list of non-staygreen genotypes classified (Fig. 1). Hence, cluster V can be categorized as non-staygreen cluster exhibiting maximum cluster mean for LSR and DSI. Intra and inter-cluster distances (table 4) were used to identify genetically diverse functional SG parents that could be recommended for incorporation in the breeding programmes for the development of SG genotypes. Inter cluster distance between Cluster III and cluster VI is maximum, hence for the development of functional SG varieties in wheat under water deficit condition, the genotypes from cluster III viz., CHIRYA7, HW2041 can be selected and crossed with cluster VI genotype viz. HW2063.

Conclusions

Staygreen trait i.e., leaf senescence rate shows positive association with relative water content, photosynthetic rate, ear weight, thousand kernel weight and grain yield. Grouping genotype for drought tolerance and staygreen trait suggested, CHIRYA7 as the most drought tolerant staygreen genotype and CBW38 as drought susceptible fast senescing genotype. D²-clustering grouped the genotypes into six clusters and cluster III genotype displayed minimum cluster mean for DSI and LSR.



Fig. 2. K Wards minimum variance dendrogram which shows the distribution of the 35 wheat genotypes under drought condition

Maximum inter-cluster distance was between Cluster III and cluster VI hence staygreen genotypes from cluster III viz. CHIRYA7 and HW2041 can be selected and crossed with cluster VI genotype i.e., HW2063 for incorporation of functional staygreen trait for development of drought tolerant genotypes. The genotypes identified will be used as donors in conventional/molecular breeding programme for the development of drought tolerant wheat genotypes.

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