Red de Excelencia Científica

FIRCME

FISIOLOGÍA DEL RENDIMIENTO Y CALIDAD PARA LA MEJORA DE CEREALES

Is stay green relevant for further raising yield in wheat and barley?

GOBIERNO DE ESPAÑA · MINISTERIO DE CIENCIA, INNOVACIÓN Y UNIVERSIDADES · AGENCIA ESTATAL DE INVESTIGACIÓN

Agencia Estatal de Investigación
Ministerio de Ciencia, Innovación y Universidades

Stay Green relationships with yield and yield components GN and GW

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From the minimum core dataset (*nucleo minimo acordado*) **c. 71 papers with data on a SG estimate and yield components**

Mutants
Isogenic lines (genes)
Cultivars
Species

Years/sites
temperatures
Water regimes
Nitrogen
Tillage system
Spray with hormones

Almost impossible to identify a single dataset without confounded effects!!!!

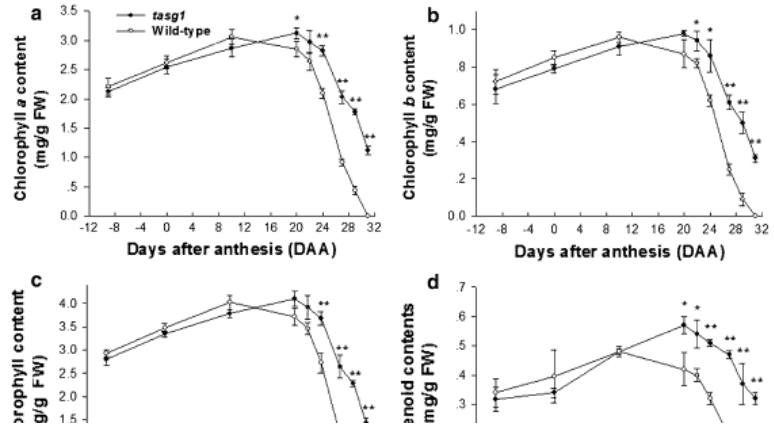


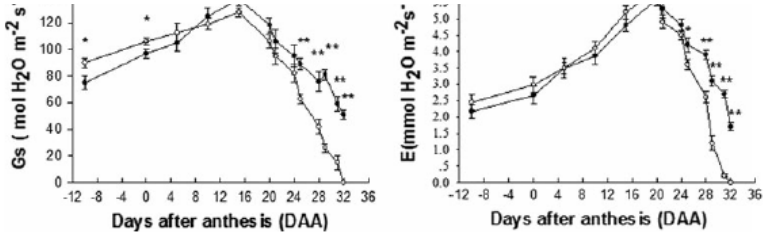
Fig. 3 Changes in a chlorophyll *a*, b chlorophyll *b*, c total chlorophyll and d carotenoid contents in flag leaves of *tasg1* and wild type during senescence (±SD). *Significant difference was noted at the 5% and **1% thresholds

Mutant for higher SG would have also affected pre-anthesis yield determination; Gains in GN more important than in GW (in fact SG mutant with lower GW !!!)

Table 1 The yield (mean ± SD) within a plot (4 m^2) and its component of *tasg1* and WT in the field

Variety	Number of seeds in each wheat spike	Number of wheat spikes in each line	1,000 grain weight (g)	Yield of each plot (kg)
Wild type	39.5 ± 4.4b	162.9 ± 19.0a	52.5 ± 0.3a	2.685 ± 0.186a
tasg1	48.8 ± 2.8a	171.5 ± 6.7a	44.8 ± 1.6b	3.000 ± 0.276a

The letters next to the values demonstrate significant difference at 0.05 thresholds



Hui et al., 2012

WT + mutant for stay green (*tasg1*) - Field Exp *tasg1*>chlorophyll duration>Pn

Another study with the same mutants (WT + mutant for stay green (*tasg1*); Field Exp. This time under 2 water regimes (CK and DS)

Again the mutant with SG improved yield (under both Control and Drought conditions) but through GN; GW was actually reduced!!!

Table 3. Kernel yields of field-grown *tasg1* and WT in 2010. Values are mean ± SE (n = 3). Different letters indicate significant differences at a 0.05 level.

Treatments	Number of kernels [spike^{-1}]	Number of spikes [m^{-2}]	Kernel mass [mg]	Yield [m^{-2}]
CK WT	39.4±1.8a	300.1±4.1a	51.7±0.7a	0.609±0.030a
DS WT	35.0±2.0c	268.2±3.5ab	50.6±1.0b	0.475±0.038b
CK *tasg1*	50.6±1.4b	302.0±4.65a	43.8±0.4a	0.667±0.044a
DS *tasg1*	44.0±2.0d	272.0±4.0a	42.5±1.0b	0.509±0.033b

Tian et al., 2012

Montazeaud et al., 2016. 3 populations, 2 seasons, Field Exp

>Stg >GW & GN but differed in heading and GN more relevant than GW

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, Turke 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 (AV 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	<0.01	<0.01	<0.01	NA	<0.01	<0.01	<0.01	<0.01	<0.05	<0.01	<0.01
	p-valY	<0.01	<0.01	<0.01	NA	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	p-valG*Y	0.31	0.83	<0.01	NA	<0.01	<0.05	0.16	<0.05	0.14	<0.05	<0.01
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-valG	<0.05	<0.05	<0.01	<0.10	<0.01	<0.01	<0.01	<0.05	<0.05	<0.05	<0.01
	p-valY	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	p-valG*Y	<0.05	0.1	<0.01	0.25	<0.01	<0.01	0.98	<0.05	<0.01	<0.01	<0.05
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	<0.01	NA	NA	<0.10	<0.01	<0.01	<0.05	<0.10	<0.05	<0.01	<0.01

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 senescen 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, degr days. NA, not available.

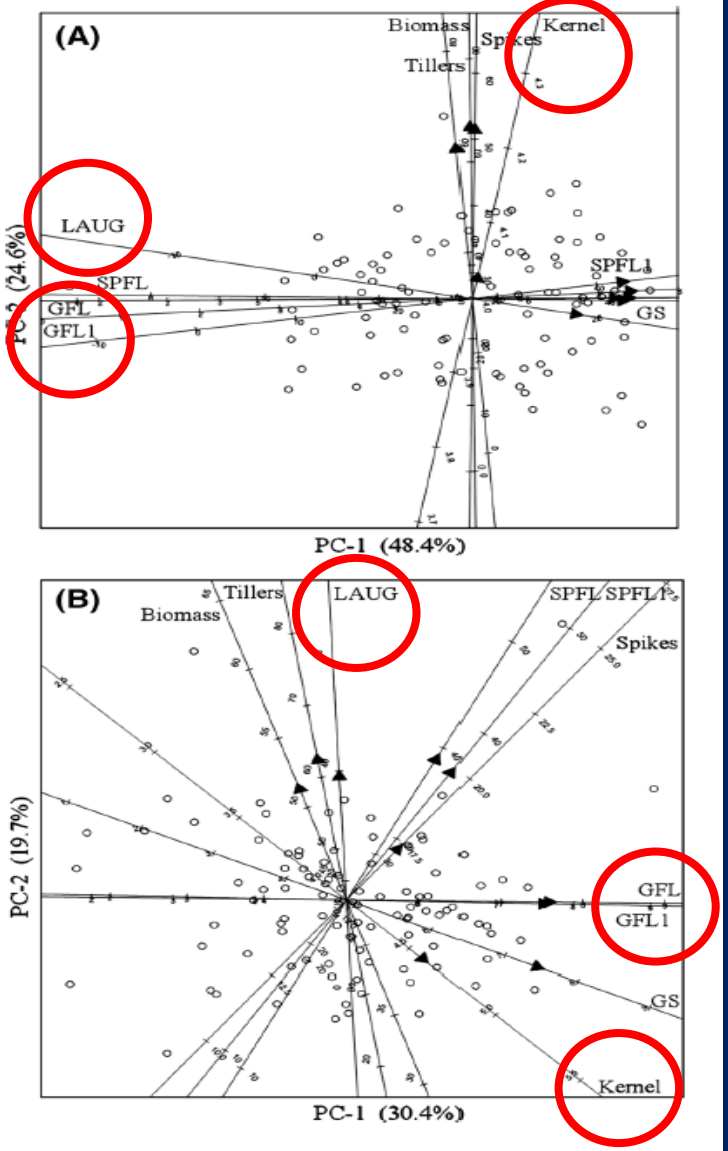


Fig. 2 Bi-plots from principal component analysis displaying the correlation between season averages of visually assessed and SPAD measured SG expression of the spike (GS), flag leaf (GFL and SPFL), first leaf under the flag leaf (GFL-1 and SPFL1-1), leaf area under green (LAUG) and key morphological traits (Biomass, 100 kernel weight (Kernel), tiller number (Tiller) and spike number (Spike)). Bi-plot (a) presents the trait relationships under terminal heat-stress, explaining 73.0 % of the variance observed, while bi-plot (b) displays the trait relationships under terminal water-stress, explaining 50.1 % of the variance observed

100 lines from DH population, heat and drought stress Growth chamber exp

Heat stress
after anthesis

Although no correlation between SG expression and anthesis date, there was variation in phenology….
In any case no relationship between SG and GW!

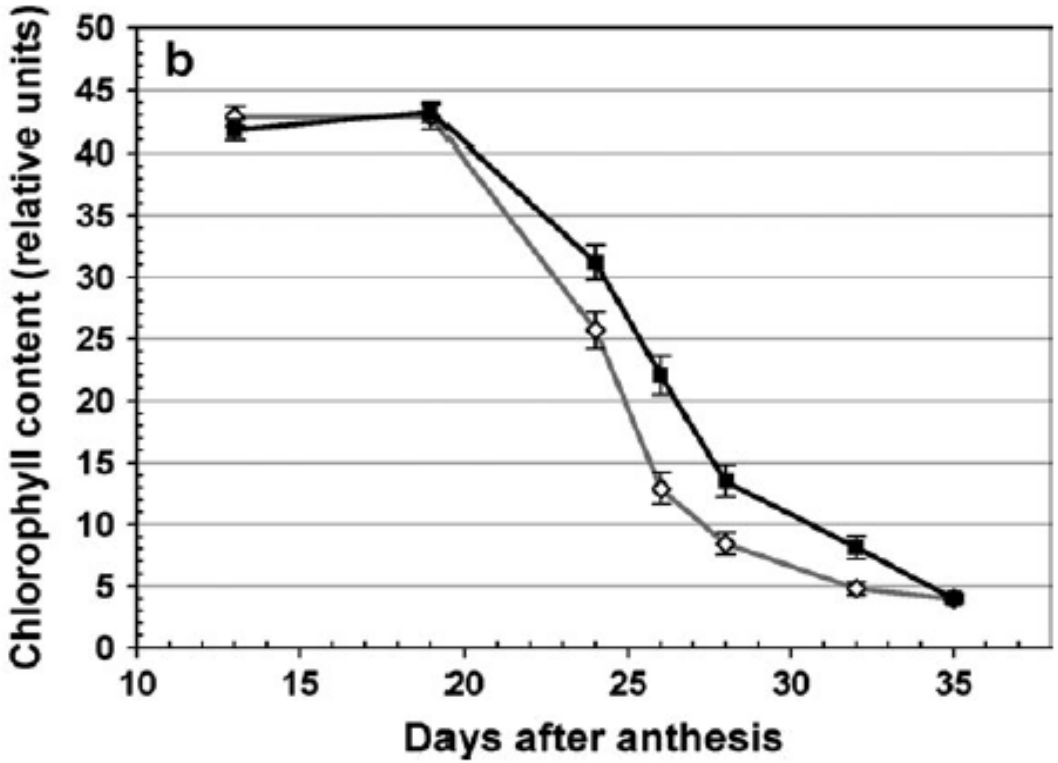
Gous et al., 2016

Water stress
After anthesis

Kernel is GW
LAUG is leaf area under Green
GFL Green flag leaf

Effect of gen *Gpc-B1 (isogenic lines) accelerating senescence,* Field exp

Uuay et al., 2006



21) and control lines (LDN and 65). RSLs 209 and 129 were similar to the
black bars), whereas RSLs 300 and 121 were similar to the high protein
b) Chlorophyll content profile (SPAD units) from 13 to 35 DAA. The grey
0 and 121) and the black line represents lines with the LDN *Gpc-B1* allele

DIC lines > senescence, >GP and < GW. Anthesis date was the same between isogenic lines

Reduced SG decreased GW in 2 backgrounds (in one with a marginal effect), but not in the other two

Uuay et al., 2006

Table 2. *Effect of* Gpc-B1 *on GPC, TKW, and total protein in isogenic lines of Anza, RSI5, U*

Differences between the means of the isogenic lines (Δ%) are reported in terms of change with respect to th allele. Significant values are presented in bold.

Line	Allele at *Gpc-B1*	GPC (%)			TKW (g)		
		Mean	Δ (%)	*P*-value	Mean	Δ (%)	*P*-value
Anza	Anza	11.74	4.98	**<0.01**	40.85	−0.06	0.98
	DIC	12.32			40.82		
RSI 5	RSI 5	13.44	15.77	**<0.01**	57.02	−4.29	**0.04**
	DIC	15.56			54.58		
UC1041	UC1041	13.30	6.93	**<0.01**	44.14	−13.02	**<0.01**
	DIC	14.22			38.40		
Kofa	Kofa	13.96	2.39	0.26	58.01	−3.59	0.10
	DIC	14.30			55.93		

Effect of temperature after anthesis, control conditions, 2 Cvs
Line with > Senescence (Xu) and > SG (Ya) (but also diffs. B4 anthesis)

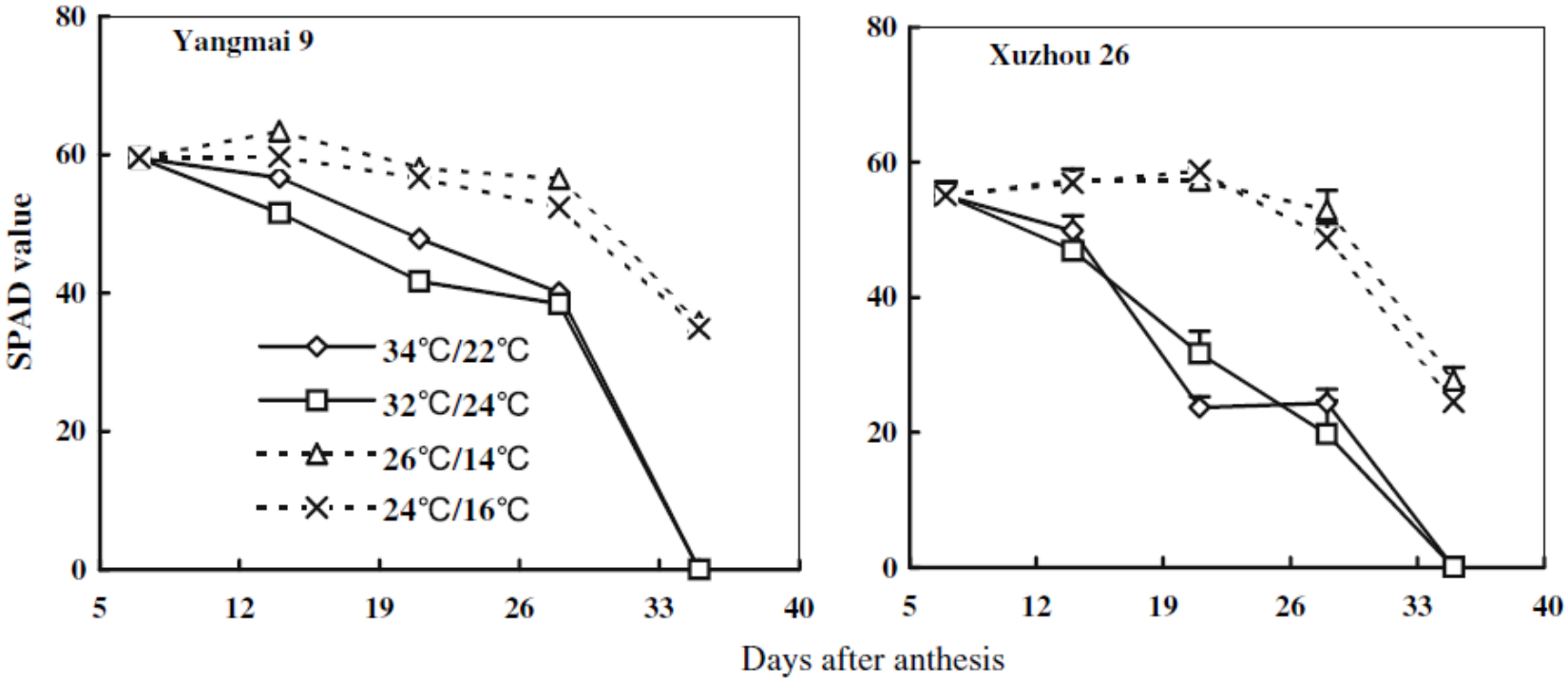


Fig. 5 Changes of SPAD value in flag leaves of two wheat cultivars under different temperature regimes

Zhao et al., 2007

Line with > Senescence (Xu) and > GW under different temperatures

Zhao et al., 2007

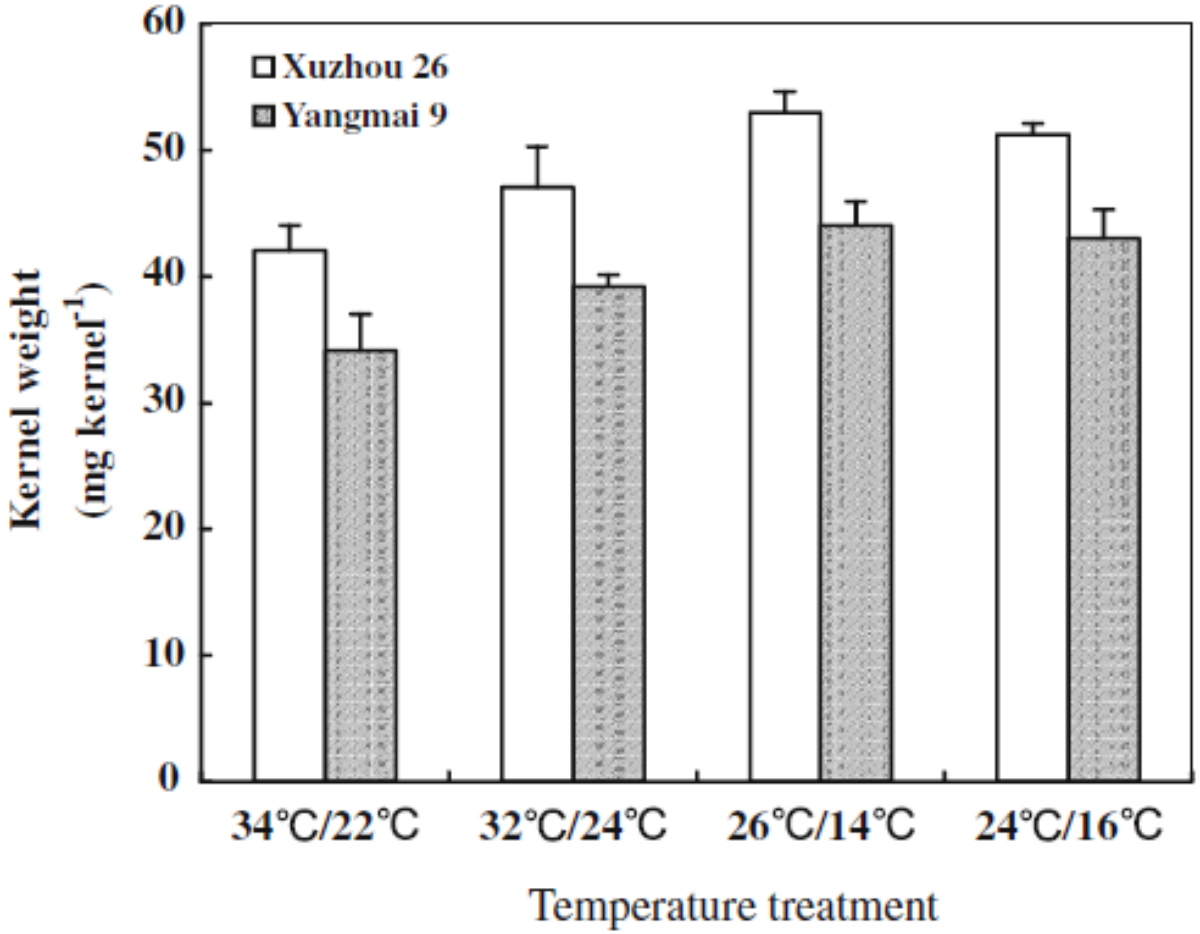


Fig. 7 Changes of grain-filling rate in two wheat cultivars under different temperature regimes

Four mutants with delayed leaf senescence were selected from seed of durum wheat mutagenized.

Changes in net photosynthetic rate, efficiency of photosystem II and chlorophyll concentration during the maturation and senescence of the flag leaves of both mutant and parental plants were determined under glasshouse conditions.

The four mutant lines maintained photosynthetic competence for longer than the parental line and are therefore functionally `stay green'.

Table 1. *Changes in the total chlorophyll content of senescing leaves of parental (cv. Trinakria) and mutant plants incubated i continuous darkness for 6 d (0 d→6 d)*

Leaves were excised and placed in the dark to induce senescence. LSD (P=0.05) = 0.236.

	Chl *a* (μg cm^{-2})			Chl *b* (μg cm^{-2})			Total Chl (μg cm^{-2})		
	0 d	3 d	6 d	0 d	3 d	6 d	0 d	3 d	6 d
Trinakria	76.2	40.1	3.3	56.1	28.4	1.2	132.3	68.5	4.5
139	75.7	55.0	34.7	54.8	33.1	17.2	130.5	88.1	51.9
142	75.1	58.0	38.4	54.9	36.1	22.0	130.0	94.1	60.4
504	76.5	60.1	38.2	55.5	35.9	20.9	132.0	96.0	59.1
196	76.1	57.8	37.8	55.2	36.0	21.2	131.3	93.8	59.0

Spano et al 2003

The mutant lines also had higher seed weights and grain yields per plant than the parental line. No indication of anthesis date

Spano et al 2003

Table 2. *Grain yields, weights and nitrogen contents of mutant and parental (cv. Trinakria) control plants*

Lines	1000 seed wt. (g)	1000 embryo wt. (g)	Total grain yield per plant (g)	N content per grain (mg)	Total N (mg g^{-1} dry wt)	Ears per plant
Trinakria	54.0	5.8	12.0	1.80	33.0	5
mutant 139	62.0	4.0	14.0	1.86	30.0	5
mutant 142	60.0	3.4	13.5	1.68	28.0	5
mutant 504	59.0	3.2	13.3	1.86	31.5	5
mutant 196	56.5	4.0	12.9	1.70	30.0	5
LSD (p=0.05)	0.80	0.50	0.20	0.21	0.17	0.10

20 Cvs of diverse origins, 3 water regimens in Field Exp

Liang et al. 2017

TABLE 3 Pearson correlations between post-anthesis physiological parameters, yield components and grain yield under the irrigated condition

	ΔCT2	A_{sat1}	A_{sat2}	A_{sat3}	S_{FL}	SPS	GNPS	SNPM	GNPM	PH	GW	BI	HD	GY
ΔCT1	0.103	0.010	−0.069	−0.032	−0.115	0.379***	0.286**	−0.163	0.104	0.425***	−0.055	0.249**	0.235*	0.381***
ΔCT2		−0.111	0.131	0.394***	−0.136	0.013	0.075	−0.074	−0.017	−0.226*	0.051	−0.009	0.037	0.562***
A_{sat1}			0.202	0.123	−0.002	0.185	0.071	0.086	0.126	0.116	−0.206	0.143	0.086	0.099
A_{sat2}				0.091	0.019	0.010	0.109	0.167	0.248**	−0.076	−0.048	0.220**	0.040	0.262**
A_{sat3}					−0.044	0.193	0.254**	0.171	0.326***	−0.062	−0.157	0.209*	0.079	0.411***
S_{FL}						−0.245**	−0.413***	0.052	−0.291**	−0.363***	0.078	−0.248**	−0.291**	−0.360***
SPS							0.621***	−0.368***	0.160	0.394***	0.015	0.294***	0.504***	0.225**
GNPS								−0.204*	0.527***	0.219**	−0.223**	0.235**	0.450***	0.421***
SNPM									0.688***	−0.299***	−0.445***	0.452***	−0.188	0.158
GNPM										−0.053	−0.520***	0.606***	0.163	0.422***
PH											0.106	0.341***	0.458***	0.001
GW												−0.064	−0.080	−0.192*
BI													0.267**	0.359***
HD														0.284**

A_{sat1}, A_{sat2} and A_{sat3}: light-saturated net carbon assimilation at anthesis, mid-grain filling and late grain filling, respectively; ΔCT1 and ΔCT2: canopy temperature depression at mid- and late grain filling, respectively; S_{FL}: flag leaf senescence; SPS: the number of spikelet per spike; GNPS: the number of grains per spike; SNPM: the number of spikes per m^2; GNPM: the number of grains per m^2; PH: plant height; GW: grain weight; BI: biomass; HD: heading date; GY: grain yield.
***, ** and * represent $p \leq .01$, $p \leq .05$ and $p \leq .1$, respectively.

No relationship significant correlation between SFL flag leaf senescence and GW, but with HD and GY under irrigation

proportion of senescent leaf area (SFL) was calculated, but no indication of total Leaf area

20 Cvs of diverse origins, 3 water regimens in Field Exp

Liang et al. 2017

TABLE 4 Pearson correlations between post-anthesis physiological parameters, yield components and grain yield under the terminal drought condition

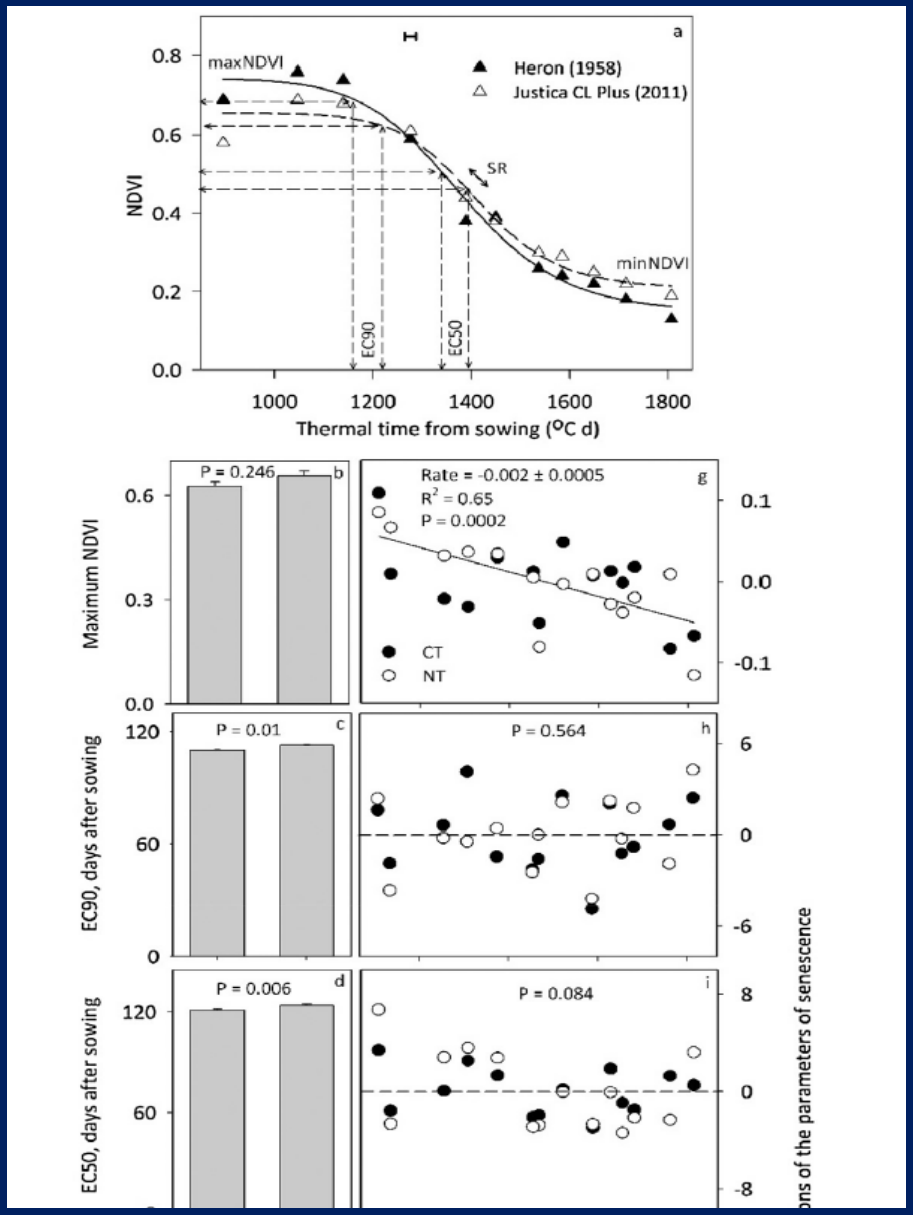
	ΔCT2	A_{sat1}	A_{sat2}	A_{sat3}	S_{FL}	SPS	GNPS	SNPM	GNPM	PH	GW	BI	HD	GY
ΔCT1	0.500***	−0.199	0.376***	0.141	−0.570***	0.226*	0.292**	−0.226*	0.011	0.218*	0.115	0.158	0.208	0.567***
ΔCT2		−0.138	0.443***	0.235*	−0.557***	0.007	0.228**	−0.158	0.007	0.044	0.431***	−0.007	0.222*	0.683***
A_{sat1}			−0.033	−0.112	0.046	0.036	0.205	−0.054	0.087	−0.156	−0.168	0.028	−0.020	0.082
A_{sat2}				0.377***	−0.373***	0.253**	0.312***	0.038	0.278**	−0.120	0.091	0.228*	0.100	0.600***
A_{sat3}					−0.325**	0.251*	0.325**	−0.184	0.009	−0.200	0.122	−0.065	0.026	0.316**
S_{FL}						−0.390***	−0.572***	0.319**	−0.135	−0.275**	−0.262**	−0.181	−0.346***	−0.629***
SPS							0.468***	−0.360***	−0.034	0.379***	0.053	0.259**	0.442***	0.156
GNPS								−0.329***	0.381***	0.003	−0.015	0.186*	0.277**	0.420***
SNPM									0.732***	−0.324***	−0.272**	0.544***	−0.399***	−0.099
GNPM										−0.277**	−0.309***	0.640***	−0.167	0.212*
PH											0.016	0.124	0.501***	0.198*
GW												−0.158	0.114	0.278**
BI													−0.015	0.198*
HD														−0.103

A_{sat1}, A_{sat2} and A_{sat3}: light-saturated net carbon assimilation at anthesis, mid-grain filling and late grain filling, respectively; ΔCT1 and ΔCT2: canopy temperature depression at mid- and late grain filling, respectively; S_{FL}: flag leaf senescence; SPS: the number of spikelet per spike; GNPS: the number of grains per spike; SNPM: the number of spikes per m^2; GNPM: the number of grains per m^2; PH: plant height; GW: grain weight; BI: biomass; HD: heading date; GY: grain yield.

***, ** and * represent $p \leq .01$, $p \leq .05$ and $p \leq .1$, respectively.

Significant correlation between SFL flag leaf senescence and GW (negative, then presumably positive between SG and GW) under manipulated terminal drought (but again with confounded effect of phenology)

14 Cvs released different eras, 2 tillage system



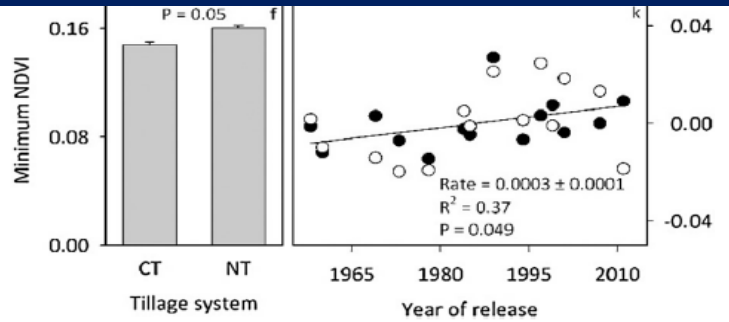
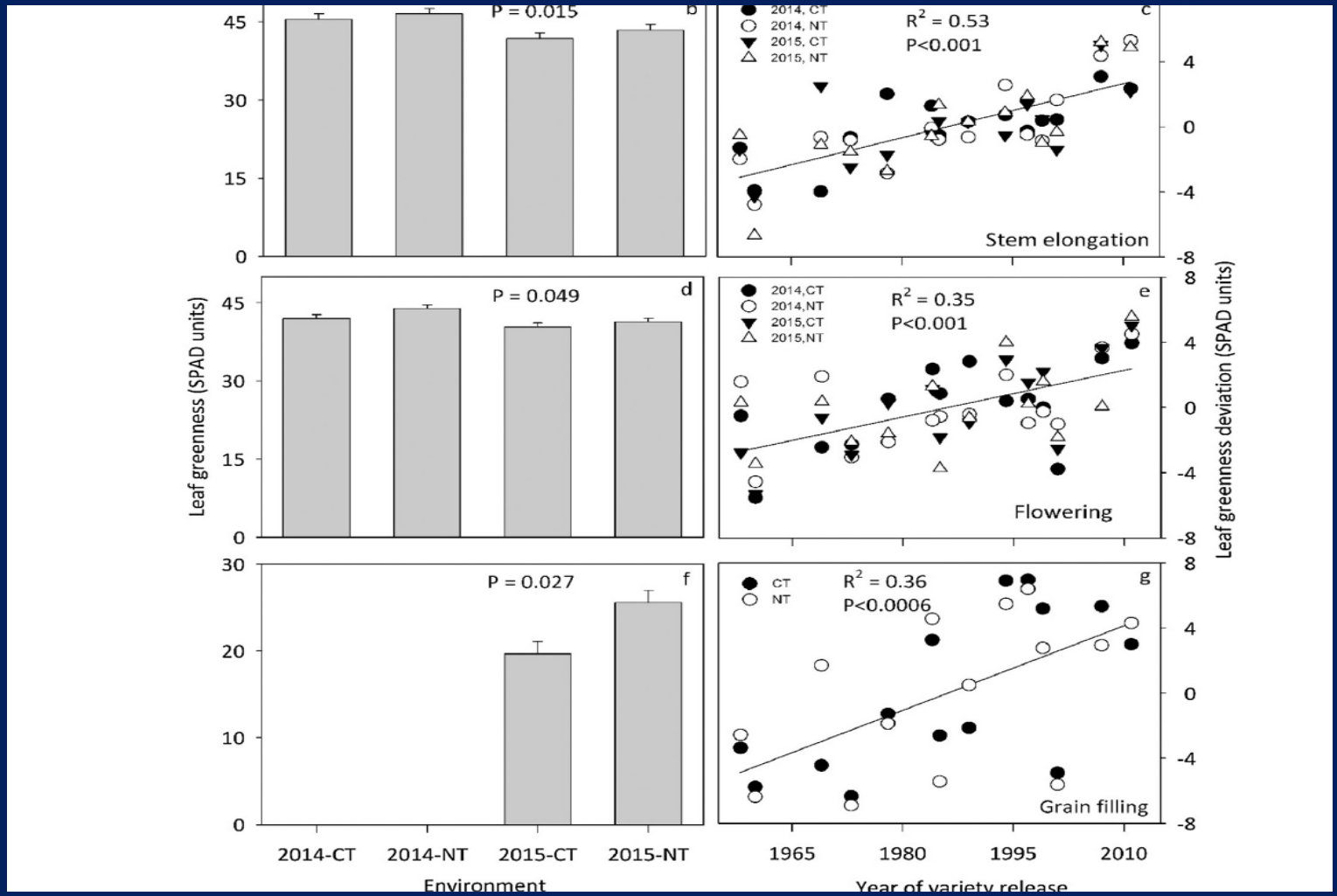


Fig. 4. An illustration of an NDVI fitted time-course for the oldest variety (1958) and a modern variety (2011) released in Australia (a). NDVI: normalised vegetative index; maxNDVI: maximum NDVI; minNDVI: minimum NDVI; EC90: onset of senescence; EC50: time to loss of 50% of maximum NDVI; SR: senescence rate. Bar shows phenostage flowering. Conventional tillage (CT) and no-till (NT) means (b–f). Error bars are 1 standard error of mean. Parameters of senescence deviations g, h, i, j and k, with the closed symbols denoting CT and the open symbols representing NT. Solid lines are linear regressions with slopes different from zero. Dashed lines denote y = 0.

Modern Cvs with similar timing of onset of senescence; but maintaining a slightly higher NDVI

14 Cvs released different eras, 2 tillage system



Modern Cvs then maintaining greeness better than old ones during grain filling, but this was also true before anthesis, and correcting by time to anthesis does not remove this difference in growth

There are a number of papers reporting relationships between SG and yield comonents

Most of them have confounded effects of phenology and or growth traits (LAI, greeness, etc) before anthesis as well

Consequently in many cases SG more related to GN than to GW!!!

In cases were confounded effects are less clear, no conclusive evidences of the relevance of SG: yes in some lines but not in others; yes in some experiments but nt in others….