

Developmental and physiological traits associated with high yield and stay-green phenotype in wheat

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Abstract. Water availability is a key limiting factor in wheat production in the northern grain belt of Australia. Varieties with improved adaptation to such conditions are actively sought. The CIMMYT wheat line SeriM82 has shown a significant yield advantage in multi-environment screening trials in this region. The objective of this study was to identify the physiological basis of the adaptive traits underpinning this advantage. Six detailed experiments were conducted to compare the growth, development, and yield of SeriM82 with that of the adapted cultivar, Hartog. The experiments were undertaken in field environments that represented the range of moisture availability conditions commonly encountered by winter crops grown on the deep Vertosol soils of this region. The yield of SeriM82 was 6–28% greater than that of Hartog, and SeriM82 exhibited a stay-green phenotype by maintaining green leaf area longer during the grain-filling period in all environments where yield was significantly greater than Hartog. However, where the availability of deep soil moisture was limited, SeriM82 failed to exhibit significantly greater yield or to express the stay-green phenotype. Thus, the stay-green phenotype was closely associated with the yield advantage of SeriM82. SeriM82 also exhibited higher mean grain mass than Hartog in all environments. It is suggested that small differences in water use before anthesis, or greater water extraction from depth after anthesis, could underlie the stay-green phenotype. The inability of SeriM82 to exhibit stay-green and higher yield where deep soil moisture was depleted indicates that extraction of deep soil moisture is important.

Additional keywords: drought adaptation, SeriM82.

Introduction

The pressure to improve yields in the rain-fed cropping environments of Australia is increasing. The growing world population and growing affluence are driving up demand for export grain. Concurrently, increasing demand for feed grain and projections of future demand for energy production are tending to drive up domestic demand. In the absence of large additional cropping areas, the only way to achieve the required increase in production is through greater production from existing areas.

The major factor limiting yield in variable rain-fed environments is water availability. The rate of genetic advance for yield in such environments has been constrained due to genotype \times environment interactions ($G \times E$), which change the rankings of genotypes from site to site and from season to season (Cooper *et al.* 2001; Richards *et al.* 2002). This is particularly true in the northern grain belt of Australia where severe climate variability occurs under the influence of the El Niño phenomenon (Stone *et al.* 1996), with great effect on wheat yield (Potgieter *et al.* 2005). The expression of physiological and morphological traits linked with high yield in the target population of environments (TPE) may be less susceptible to

environmental influences than yield *per se*. It has been proposed that selection for such traits can improve the rate of yield gain in the face of $G \times E$ (Jackson *et al.* 1996; Hammer *et al.* 2002, 2005). An example of this approach is selection for the stay-green trait to improve drought adaptation and yield stability of sorghum in the northern grain belt of Australia (Borrell *et al.* 2006). There are several other instances where physiological traits have been identified and used as secondary selection criteria in breeding programs (Fischer *et al.* 1989; Fukai *et al.* 1999; Reynolds *et al.* 2000a; Richards *et al.* 2002). There are also potential interactions with management practices leading to genotype \times management \times environment interactions ($G \times M \times E$) which, while adding a further layer of complexity, also generate significant opportunity for more rapid yield advance (Hammer and Jordan 2007).

The CIMMYT wheat genotype, SeriM82, is widely adapted to rain-fed spring-wheat regions of the world but has not been widely grown in Australia due to disease susceptibility and aspects of flour quality (Sivapalan *et al.* 2000, 2001). In both international trials and Australian trials, SeriM82 produces higher yields than cv. Hartog, itself derived from a CIMMYT line, Pavon 76. In contrast to SeriM82, Hartog is well adapted and

widely grown in the northern Australian grain belt. The yield of SeriM82 was on average 3.5% greater than that of Hartog in a range of north African and west Asian environments (Sivapalan *et al.* 2001) and 4.5% greater in a range of environments within Australia (Sivapalan *et al.* 2000). Both genotypes ranked higher in subtropical than Mediterranean environments in Australia and could be considered good indicator lines for performance in subtropical environments (Sivapalan *et al.* 2001). In multiple-environment trials in the sub-tropical northern grains belt of Australia over several seasons, yield of SeriM82 was 12% greater than that of Hartog in both water-limited and non-limited conditions (Cooper *et al.* 1994, 2001; Peake *et al.* 1996; Peake 2003). Pattern analysis of MET data for yield and grain protein concentration indicated that while SeriM82 exhibited high positive scores for yield, the scores for grain protein concentration tended to be low to intermediate (Cooper *et al.* 2001). Stable carbon isotope analyses indicate that neither SeriM82 nor Hartog have high transpiration efficiency of the type exhibited by Quarrion (Condon *et al.* 1990; Condon and Richards 1992; Fischer *et al.* 1998; Rebetzke *et al.* 2002). The genotypes differ in dwarfing genes, with SeriM82 having Rht-B1b (Rht1) and Hartog Rht-D1b (Rht2; Reynolds *et al.* 2000b; Trethowan *et al.* 2001).

Adaptations of both above- and below-ground processes are likely to influence response to water-limited environments in the Australian northern grain belt. Due to the summer-dominant rainfall pattern, winter crops rely heavily on stored soil moisture in the deep clay soils. This can lead to severe terminal moisture stress in seasons with little in-crop rainfall. Little is known about the morphological and physiological differences between SeriM82 and Hartog plants under field conditions, particularly in Australian environments. However, in a recent detailed study on root growth and water extraction pattern of single plants grown in large root-observation chambers, Manschadi *et al.* (2006) demonstrated that, compared with Hartog, SeriM82 possesses a more compact, uniform, and deep root architecture, which appears to increase access to water from the deeper soil layers during the grain-filling period.

Attempts to breed elite wheat varieties with superior yield to Hartog by making crosses between SeriM82 and Hartog have been unsuccessful to date. Research using a population of recombinant inbred lines developed from one such cross has indicated that the high yield is not associated with the 1BL/1RS translocation carried by SeriM82 (Peake *et al.* 1996). Further, the superior yield of SeriM82 over Hartog is in part due to positive epistatic genetic effects (Peake 2003). These results suggest that high yield in SeriM82 is under complex genetic control.

The objective of this research was to elucidate the physiological basis for the yield advantage of SeriM82 in the field in the Australian northern grain belt. The performance of SeriM82 was compared with that of Hartog at a common site over several seasons. Water availability varied from non-limited fully irrigated conditions to severe pre- and/or post-anthesis drought. Detailed observations were taken on developmental and physiological attributes both above and below ground. The fact that the high yield of SeriM82 is under complex genetic control suggests that there could be several underlying physiological processes, some of which may have only small effects. In order to maximise the chances of detecting such

differences by taking detailed measurements, we restricted studies to a single site with a well characterised, common soil type and used a single conventional management strategy.

Methods

Plant material

Seed of wheat genotypes SeriM82 and Hartog was obtained through the Queensland Department of Primary Industries and Fisheries, Northern Wheat Improvement Program. Seed from a common source was used coming from plants grown in fully irrigated increase rows at Kingsthorpe in the season before each trial. Seed mass and germination rate were determined for each seed sample before sowing.

Field site and environment conditions

During the winter seasons of 2003, 2004, 2005, and 2006, field experiments were conducted at the Queensland Department of Primary Industry and Fisheries research farm at Kingsthorpe (27.51°S, 151.78°E; 442 m a.s.l.). The soil is a Craigmore (Harris *et al.* 1999) black Vertosol (Isbell 1996), characterised as a deep, coarse, self-mulching, black cracking clay.

In all experiments, seed was sown at a row spacing of 25 cm, with a target population density of 100 plants/m² in a complete randomised design.

In 2003, wheat genotypes Hartog and SeriM82 were grown in 1 m by 6 m plots with 4 replicates per genotype per treatment. The 2 treatments consisted of plots that were either irrigated to minimise moisture stress (irrigated treatment) or were not irrigated and had rainfall excluded from the time of head emergence (79 days after emergence, DAE) until maturity (rain excluded treatment; Table 1). Shelters of 7 m by 6 m by 6 m covered with translucent plastic that transmitted >80% of incident sunlight were moved to cover the plots during periods of rain and were removed when rain was not imminent.

In 2004 and 2005, wheat genotypes Hartog and SeriM82 were sown in plots of 1.95 by 8 m and 1.95 by 6 m, respectively, with 3 replicates in 2004 and 4 replicates in 2005, under natural rain-fed conditions. In 2006, Hartog and SeriM82 were sown in 2 by 6 m plots with 4 replicates into an area where soil moisture had been depleted to a depth of 1.5 m by a dense crop of forage sorghum in the previous summer.

All replicates were used for detailed measurement in 2003 and 2004. In 2005 and 2006, however, only 3 of the 4 replicates were instrumented for soil moisture measurements, and in 2005, only 3 were used for consecutive biomass measurements between anthesis and maturity. Hence, to facilitate comparisons, all data for 2005 and 2006 are presented only for the 3 replicates where detailed measurements were taken.

Measurement of developmental and physiological traits

Detailed observations of the phenology of the crop (Zadoks *et al.* 1974) were taken throughout the season. Regular quadrat harvests of not less than 0.5 m of crop row were taken periodically to determine leaf area, and number and dry mass (oven-drying at 65°C for 72 h) of leaves, stems, and spikes. For all experiments, biomass samples were taken at anthesis and maturity, while in 2004 and 2005, further samples were taken during the grain-filling period. At maturity, grain yield, grain number, and grain mass were recorded using quadrat harvests of

Table 1. Planting time, plant density (plants/m²), days after emergence (DAE) to anthesis, days to maturity, and moisture availability (mm) for wheat lines Hartog/SeriM82 in trials at Kingsthorpe in the Australian northern grain belt

Year	Treatment	Plant date	Density	Anthesis DAE	Maturity DAE	Potentially available moisture		
						Initial soil ^A	Rain plus irrigation Pre anthesis	Post anthesis
2003	Irrigated	25 June	72/66	83/84	132/137	137/130	179/179	98/98
	Rain excluded ^B	25 June	67/59	83/84	<118/118	138/131	29/29	–
2004	Rain-fed	23 June	71/74	93/93	114/123	214/214	36/36	20/20
2005	Rain-fed	11 July	120/102	73/74	102/109	222/207	26/26	90/104
2006	Profile depleted ^C	9 June	85/76	91/94	121/121	74/73	41/41	41/41

^APAWC to 1.5 m for 2003 and 2004, to 1.9 m for 2005, and to 0.7 m for 2006.

^BRain excluded post anthesis at ~79 DAE for 2003 only.

^CInitial soil moisture depleted by a previous summer crop.

2 m of crop row (0.5 m²) in 2004 and 2005, and 1 m of crop row (0.25 m²) in 2003, as well as with a small plot harvester in 2006.

Leaf greenness, a surrogate measure of leaf chlorophyll content, was monitored using a Minolta SPAD 502 (Konica Minolta, Hong Kong). Flag leaves from an equal number of plants per plot (ranging from 4 in 2006 to 8 in 2004) were tagged shortly after they were fully expanded. SPAD measurements were taken at the central point of each flag leaf at each time of measurement. The data are expressed in arbitrary SPAD units with values from 55 to 65 representing the maximum values typical for newly fully expanded leaves and values below 10 typical of fully senesced leaves.

Measurement of soil moisture

Aluminium soil access tubes were fitted mid-way between the central crop rows and near the centre of each plot to allow water extraction to be followed using a neutron moisture probe (503 HR Hydroprobe, CPN International, CA, USA). Measurements were taken at 0.20-m increments from 0.10 m below the soil surface to a depth of 1.50 m in 2003 and 2004 and to a depth of 1.90 m in 2005 and 2006. Measurements were taken at weekly intervals whenever possible in 2003 and 2005. In 2004 and 2006, measurements were taken at planting, near anthesis, and at maturity. Weekly measurements were also taken during the grain-filling period in 2004.

Analysis of soil cores taken at the time of planting revealed that soil nitrogen (N) and phosphorus (P) were at luxury levels and that the numbers of parasitic nematodes *Pratylenchus neglectus* and *P. thornei* were low in all seasons.

Data analysis

All statistical analyses of data were conducted using the GENSTAT software system.

Results

Variation in moisture availability

By exploiting natural variation in rainfall amount and timing combined with manipulation of irrigation it was possible to generate a range of moisture stress environments over several seasons at a single site (Table 1). This allowed responses to moisture stress of the 2 genotypes to be compared while minimising confounding effects such as variation in soil type. The black Vertosol soil is representative of the more productive

soils occurring in the northern grain belt, being over 2 m in depth with high water-holding capacity. The range of moisture stress environments generated in 2003, 2004, and 2005 could be considered representative of the range commonly affecting winter cereals on this soil type in the northern grain belt. The irrigated environment in 2003 was representative of years where the plants experience little moisture stress due to periodic in-crop rainfall (Table 1). The 'rain excluded' treatment of 2003 and the rain-fed environment of 2004 represented situations of severe terminal moisture stress where plants received less than 60 mm of in-season rainfall. In these treatments the plants started with sufficient stored soil moisture to allow development with moderate stress up until anthesis but experienced increasing moisture stress from anthesis until maturity. In the rain-fed environment of 2005, plants also started with sufficient soil moisture, but after a period of moisture stress around anthesis, received sufficient moisture from precipitation events starting during the mid grain-filling period and continuing until maturity (Table 1). A further environment was generated in 2006 when forage sorghum was used to deplete soil moisture in the summer before sowing the crop. Rainfall after the sorghum crop provided 74 mm of plant-available soil moisture for plant establishment, with a further 41 mm of irrigation applied immediately following planting (Table 1). This was not sufficient moisture to avert the onset of stress well before anthesis. A further 41 mm of irrigation was added near anthesis to allow the plants to develop through to maturity but with moisture stress increasing again during the grain-filling period. The levels of moisture stress induced in 2006 represent an extreme not usually observed for crops on deep Vertosol soils in the northern grain belt because growers would not normally plant into such a dry soil profile. A range of responses in yield, green leaf area dynamics, and biomass accumulation were observed for the 2 genotypes under the various moisture stress environments.

Yield under varying environmental conditions

The yield of SeriM82 was significantly greater than that of Hartog in all years and environments except 2006 (Table 2). The yields ranged from a maximum of 780 g/m² for SeriM82 in the fully irrigated environment of 2003 down to 207 g/m² for Hartog in 2006. The yield advantage to SeriM82 of 28% was greatest for the irrigated environment of 2003 and was least in

Table 2. Yield (g/m²), grain mass (g/1000), and grain number (10³/m²) of wheat varieties SeriM82 and Hartog in trials at Kingsthorpe in subtropical Australia

Difference (%) is calculated as the value for SeriM82 minus the value for Hartog over the value for Hartog multiplied by 100. *Values for varieties are significantly different within treatments (*P* < 0.05)

Year	Treatment	Yield			Grain mass			Grain number		
		Hartog	Seri	Diff.	Hartog	Seri	Diff.	Hartog	Seri	Diff.
2003	Irrigated	610	780	28*	39	44	22*	16	18	13
	Rain excluded ^A	260	320	14*	25	29	16*	11	10	-9
2004	Rain-fed	390	460	18*	23	26	13*	17	18	6
2005	Rain-fed	280	340	20*	23	31	35*	12	11	-8*
2006	Profile depleted ^B	207	219	6	22	26	18	9.6	8.3	-14

^ARain excluded post anthesis at ~79 DAE for 2003 only.

^BInitial soil moisture depleted by a previous summer crop.

2006. SeriM82 had significantly higher grain mass in all seasons except in 2006. The percentage advantage to SeriM82 in mean grain mass was greatest in 2005 at 35%. In contrast to grain mass, the grain number differed significantly between genotypes in only one season, 2005. In 2005 the grain number of SeriM82 was 8% less than that of Hartog. The harvest index of SeriM82 tended to be higher than that of Hartog and was significantly higher in 2004 and 2005 (*P* < 0.05, *n* = 3; data not shown).

Crop development

SeriM82 has been observed to reach anthesis up to 7 days later than Hartog when planted in April, May, or early June (data not shown). This suggested that under more favourable growing conditions, SeriM82 may be able to achieve greater biomass and yield than Hartog due to a longer growing season. To minimise differences in crop phenology, experiments in 2003, 2004, and

2005 were sown in late June or early July (Table 1). This had the desired effect of coordinating the 50% anthesis dates (Zadoks stage 65) such that SeriM82 and Hartog were no more than one day apart (Table 1). Late June and early July sowing dates are within the normal planting window for the region. Planting the crop in early June of 2006 led to SeriM82 reaching anthesis 3 days later than Hartog (Table 1).

Leaf area dynamics, biomass accumulation, and partitioning

The most striking differences in leaf area were observed post anthesis. In both years where leaf area index (LAI) was determined by sampling between anthesis and maturity (2004 and 2005), SeriM82 maintained a higher green leaf area for a longer period after anthesis (Fig. 1). Differences in leaf area before and at anthesis were less notable. There was no significant difference between genotypes in the maximum LAI reached near

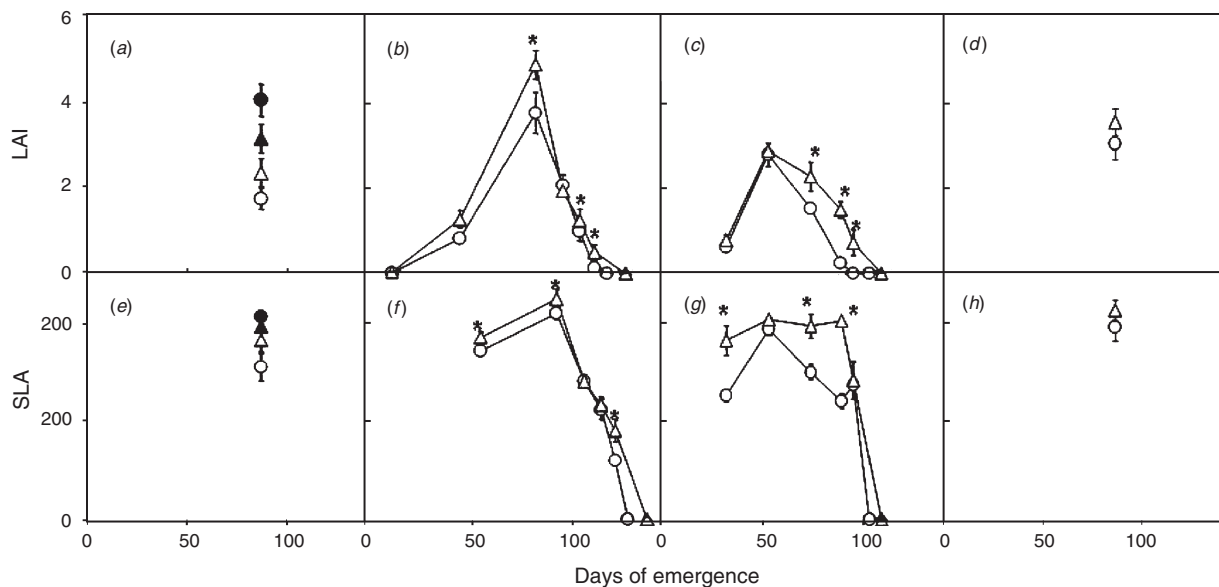


Fig. 1. (a-d) Leaf area index (LAI; m²/m²) and (e-h) specific leaf area (SLA; cm²/g dry weight green leaf) for wheat genotypes Hartog (circles) and SeriM82 (triangles). Plants were grown under non-limiting irrigated conditions in 2003 (closed symbols; a and e) or under various conditions of moisture stress (open symbols) in the growing seasons of (a, e) 2003, (b, f) 2004, (c, g) 2005, and (d, h) 2006. Error bars represent the standard error of the mean and in some cases are obscured by symbols. Asterisks indicate that means for genotypes within treatments were significantly different (*P* < 0.05).

anthesis in 2003, 2005, and 2006 (Fig. 1). In 2004, SeriM82 had a higher LAI of $4.89 \text{ m}^2/\text{m}^2$ at anthesis than Hartog's of $3.76 \text{ m}^2/\text{m}^2$ (Fig. 1).

Measurements of flag leaf greenness using the Minolta SPAD meter confirmed the ability of SeriM82 to maintain green leaf area for longer during the grain-filling period in 2004 and 2005 and gave additional information for 2003 and 2006 (Fig. 2). Measurements in 2004 and 2005 agree with destructive LAI and biomass measurements, indicating that SeriM82 maintained a higher green leaf area than Hartog for a longer period following anthesis (Figs 1, 2). This demonstrates that tracking the greenness of the flag leaves gave a useful indication of the relative ability of the genotypes to maintain green leaf area. Measurements in 2003

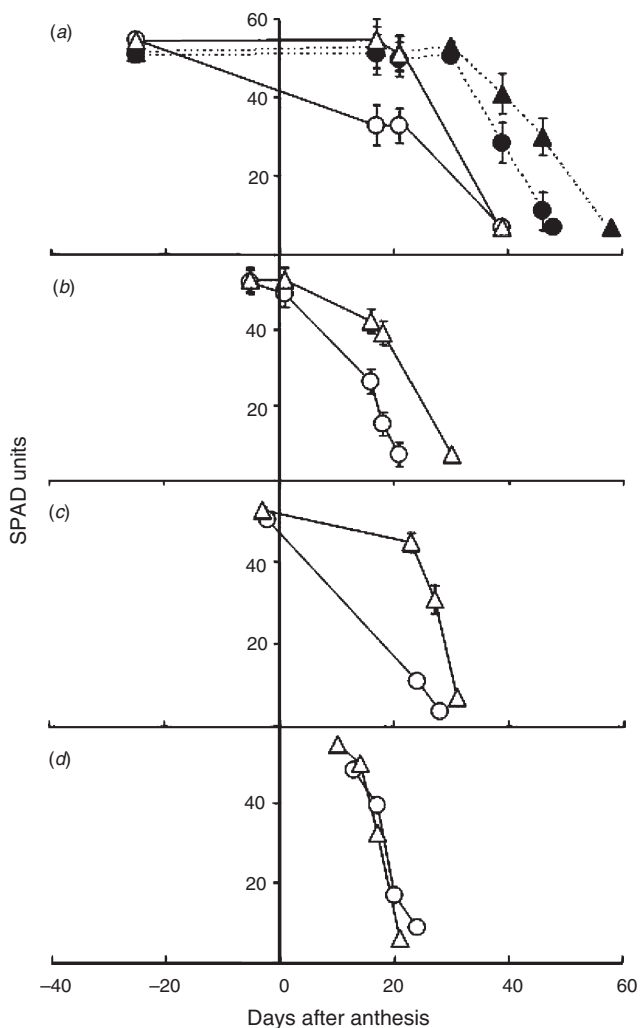


Fig. 2. Flag leaf greenness measured using a Minolta SPAD 502 expressed in arbitrary SPAD units for Hartog (circles) and SeriM82 (triangles) in (a) 2003 (closed symbols represent the irrigated treatment and open symbols the rain excluded), (b) 2004, (c) 2005, and (d) 2006. Error bars represent the standard error of the means for 4 replicates in 2003 and 3 replicates in 2004 and 2005 and are in some cases obscured by symbols. Data for 2006 are the means for a single plot without error bars. Measurements for all replicates 111 DAE in 2006 confirm that data for the single replicate presented are representative.

indicate that SeriM82 was able to maintain greater green leaf area for longer during the grain-filling period in both the irrigated and rain-excluded treatments (Fig. 2). However, in the absence of deep soil moisture in 2006, SeriM82 was not able to maintain green leaf area longer than Hartog (Fig. 2).

Accumulation in green leaf mass up until anthesis was similar for both genotypes in 2003, 2005, and 2006 (Table 1, Fig. 3). In contrast, when green leaf mass was measured during the grain-filling period (2004, 2005), SeriM82 retained a greater proportion, reflecting the retention of more green leaf area during this period (Figs 1–3). The specific leaf area of SeriM82 was significantly higher than that of Hartog in 2004 and 2005 ($P < 0.05$; Fig. 1). SeriM82 accumulated more green leaf mass ($0.22 \text{ kg}/\text{m}^2$; Fig. 3) up to anthesis in 2004 than Hartog ($0.18 \text{ kg}/\text{m}^2$). However, this was not reflected in higher stem, spike, or total biomass in SeriM82 compared with Hartog in that year (Fig. 3).

Under water limitation in 2004 and 2005, total above-ground biomass accumulation was similar for the 2 genotypes up until the time that Hartog reached maturity (Table 1, Fig. 3). However, as SeriM82 reached maturity later than Hartog in 2004 and 2005, it continued to accumulate biomass for several days longer than Hartog (Table 1, Fig. 3). In contrast to these moisture-limited treatments, under non-limiting moisture conditions in the irrigated treatment of 2003, total biomass of SeriM82 increased more rapidly than of Hartog from anthesis to maturity ($P = 0.005$, $n = 4$) and was higher at maturity ($1.63 \text{ kg}/\text{m}^2$ SeriM82, $1.34 \text{ kg}/\text{m}^2$ Hartog; $P = 0.039$, $n = 4$; Fig. 3). Accumulation of biomass in the spikes follows a similar pattern to that observed for total biomass. A similar accumulation pattern was observed between genotypes up until the maturity of Hartog in water-limited environments in 2004, 2005, and in the rain-excluded treatment in 2003, but continuing for several days longer in SeriM82 than in Hartog in 2004 and 2005 (Table 1, Fig. 3). Spike biomass of SeriM82 was higher at maturity for the irrigated treatment of 2003 ($1.03 \text{ kg}/\text{m}^2$ SeriM82, $0.80 \text{ kg}/\text{m}^2$ Hartog; $P < 0.001$, $n = 4$; Fig. 3). The rate of grain filling was measured in detail in 2004 (Fig. 4), when little difference was observed between the 2 genotypes under severe terminal drought. SeriM82 achieved higher grain mass than Hartog in 2004 because it reached maturity 9 days later and so had a longer period in which to accumulate biomass in the grain (Table 1). The maximum rates of grain filling occurring between 101 and 108 DAE, were 1.1 ± 0.1 and $1.2 \pm 0.1 \text{ mg}/\text{kernel}\cdot\text{day}$ ($n = 3$) for Hartog and SeriM82, respectively.

Loss of biomass from the stems between anthesis and maturity was similar for the 2 genotypes in 2003, 2004, and 2005 (Table 1, Fig. 3). However, in 2006 the loss of biomass from stems of SeriM82 was greater than that of Hartog ($P = 0.006$, $n = 3$; Fig. 3).

Soil moisture extraction

There was no significant difference detected between genotypes in either the maximum depth of soil moisture extraction (e.g. Fig. 5) or the net moisture extracted (Table 3). Despite detailed and frequent measurements during the course of each season, no consistent differences between genotypes could be detected in moisture extracted from any

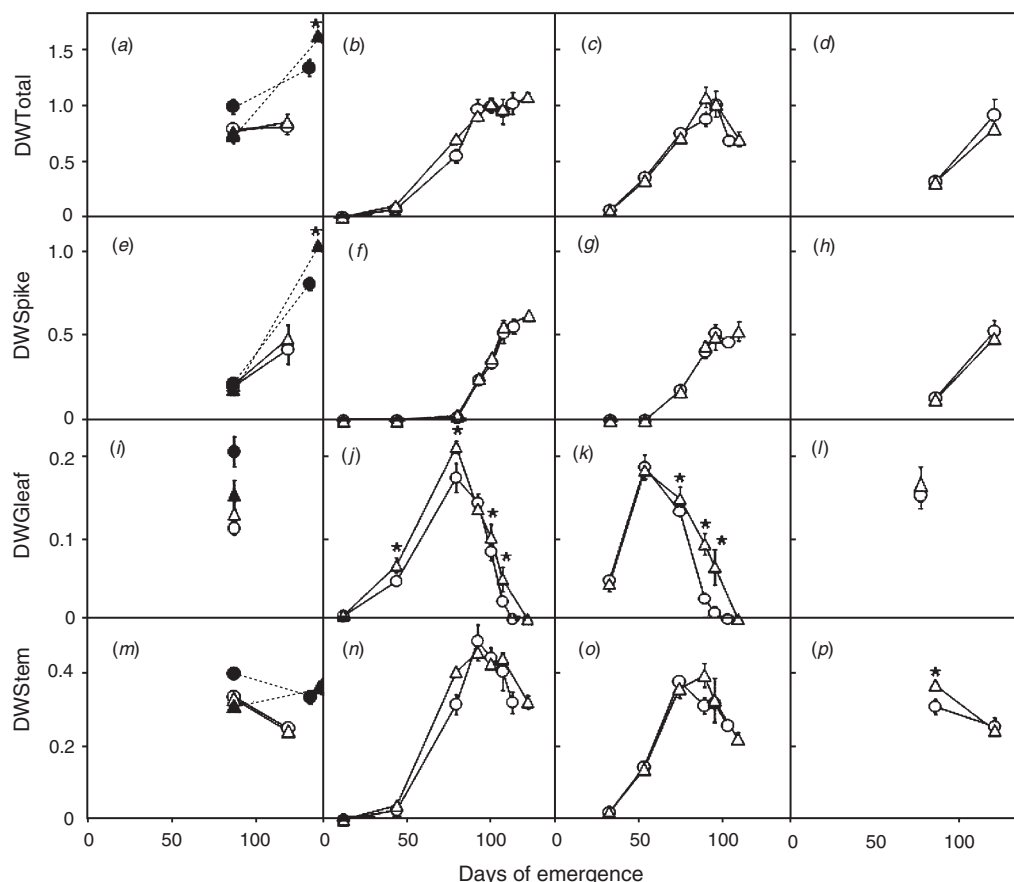


Fig. 3. (a–d) Total above-ground biomass (DWTTotal; kg/m²), (e–h) spike biomass (DWSpike; kg/m²), (i–l) green leaf biomass (DWGleaf; kg/m²), and (m–p) stem biomass (DWStem; kg/m²) for wheat genotypes Hartog (circles) and SeriM82 (triangles). Plants were grown under non-limiting irrigated conditions in 2003 (closed symbols) and under various conditions of moisture stress (open symbols) in the growing seasons of (a, e, i, m) 2003, (b, f, j, n) 2004, (c, g, k, o) 2005, and (d, h, l, p) 2006. Error bars represent the standard error of the means and in some cases are obscured by symbols. Asterisks indicate that means for genotypes within treatments were significantly different ($P < 0.05$).

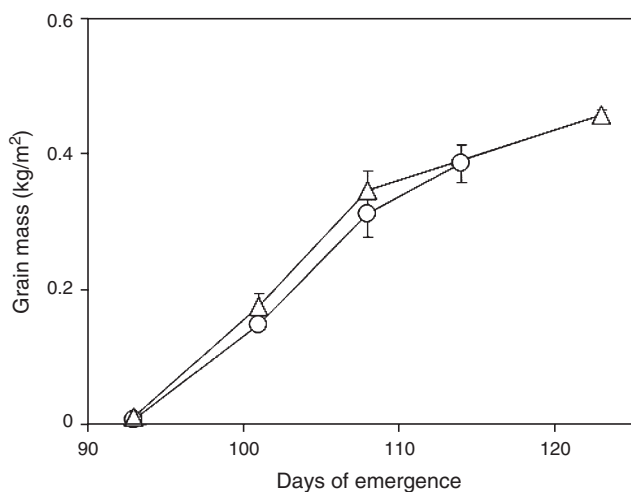


Fig. 4. Dry mass of grain per unit area for wheat genotypes SeriM82 and Hartog from the time of anthesis at 93 days after emergence up until maturity for plants exposed to severe terminal moisture stress in 2004. Values represent the mean and standard error for 3 replicates.

individual soil layer. Data for several time points in 2005 are presented as a typical example (Fig. 5). In non-irrigated treatments in 2003 and 2004, both genotypes extracted soil moisture to near the lower limit of extraction to a depth of 1.5 m by the time of anthesis. In 2006 the soil moisture was depleted to a depth of 1.5 m by a previous summer crop, and subsequent rainfall and irrigation did not penetrate more than 0.7 m. The roots of both genotypes reached a maximum depth of 0.7 m in 2006 and again there was no significant difference in total soil moisture extracted. SeriM82 did extract more moisture deep in the profile where rain-exclusion shelters were installed in a single plot of each genotype in 2005 (data not shown). However, the lack of replicate shelters precludes statistical comparison between genotypes in the sheltered plots in that experiment.

As SeriM82 exhibited higher yield than Hartog with no detectable difference in soil moisture extraction in 2003, 2004, and 2005, the mean water-use efficiency (WUE) of SeriM82 appears to be higher than that of Hartog, although the differences between genotypes in each environment are not statistically significant ($P > 0.05$; data not shown). Further

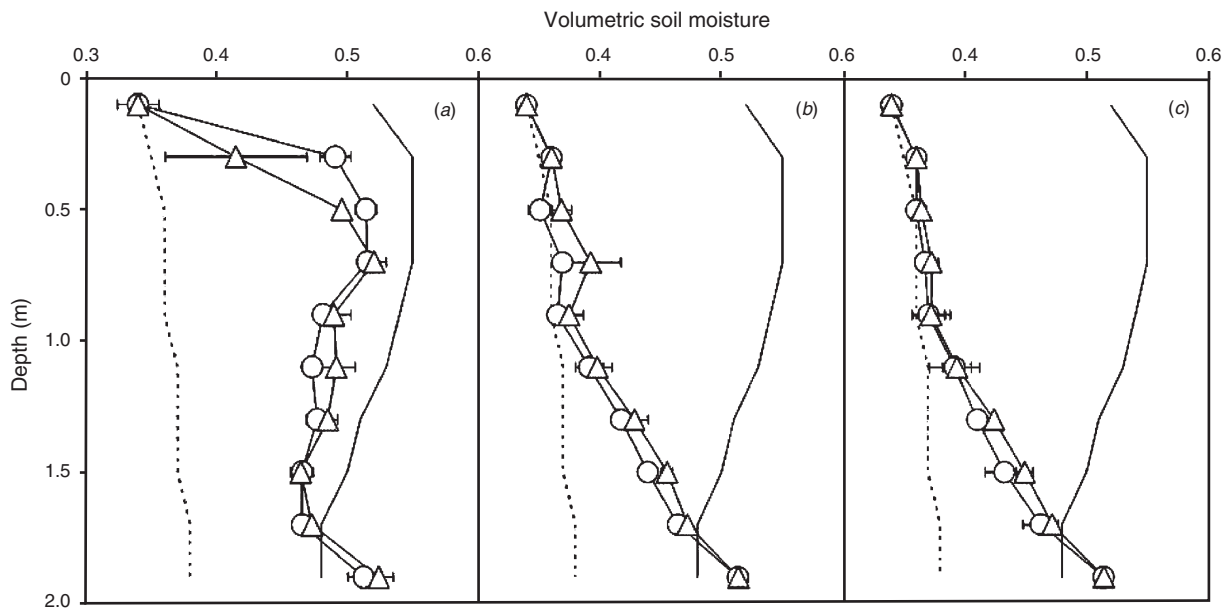


Fig. 5. Volumetric soil moisture beneath crops of wheat genotypes Hartog (circles) and SeriM82 (triangles) at (a) 7, (b) 81, and (c) 95 days after emergence at Kingsthorpe in 2005. The solid line without marker symbols represents the drained upper limit and the hatched line represents the theoretical lower limit of extraction to -1.5 atmospheres soil water potential. Values represent the mean and standard error for 3 replicates of the volumetric soil moisture content in 0.20-m soil layers plotted at the maximum depth for each layer.

experiments using lysimeters will be required to test for and to quantify any differences in WUE between these cultivars.

Discussion

Yield, development, leaf area, and biomass accumulation

The fact that the yield of SeriM82 was significantly higher than that of Hartog in all years and environments except 2006 agrees with previous multiple-environment field studies where SeriM82 exhibited higher yield than Hartog in a wide range of locations and seasonal environments in the northern grain belt (Peake *et al.* 1996; Cooper *et al.* 2001; Peake 2003). The yield advantage observed in our experiments could not be attributed to differences in phenology. The 2 genotypes reached anthesis within one day of each other in 2003, 2004, and 2005 when yield differences were significant (Tables 1, 2).

Data for yield components suggest that higher grain mass is an important factor in determining the higher yield of SeriM82 than of Hartog (Table 2). The yield of SeriM82 was not only greater than that of Hartog in 2003 and 2004 when there was no significant difference in grain number, but also in 2005 when SeriM82 had a lower grain number than Hartog (Table 2). Although grain number differed significantly only in 2005, the data for other years do suggest some trends in the relationship between grain number and the yield difference between SeriM82 and Hartog. Data for the irrigated treatment of 2003 suggest that higher grain number may contribute to the higher yield of SeriM82 where moisture availability is non-limiting (Table 2). Conversely, there is some suggestion of a tendency towards lower mean grain number, and a decrease in the relative yield advantage of SeriM82 over Hartog in the more moisture-stressed environments in 2006 and the rain-excluded environment in 2003. It is noteworthy that the

only environment where the yield of SeriM82 was not significantly higher than Hartog ($P = 0.773$, $n = 3$) was in 2006 where moisture stress was greatest, particularly pre-anthesis (Tables 1, 2). It is likely that in 2006, plants experienced more severe moisture stress early in the season (pre-anthesis) than in the other environments. The importance of grain mass in determining the yield advantage of SeriM82 over Hartog is in agreement with reports for stay-green *v.* senescent wheat near-isogenic lines in Brazil (Silva *et al.* 2003). However, this contrasts with the yield difference reported for a Seri/Babax recombinant inbred population where grain number had more influence than grain mass (Olivares-Villegas *et al.* 2007).

The ability of SeriM82 to maintain green leaf area longer after anthesis (Figs 1–3), and to continue to accumulate biomass later in the season (Fig. 3), was a major contributor to the higher grain mass and yield of SeriM82 (Table 2, Fig. 4). The similar rate of grain fill in 2004 (Fig. 4) and of increase in spike mass in all other moisture-stressed environments (Fig. 3) indicates that the greater grain mass of SeriM82 is due to the ability to prolong grain filling rather than an ability to accumulate biomass more rapidly under moisture limitation (Table 1, Figs 1–3). However, the increase in spike biomass observed in the irrigated treatment in 2003, suggests that SeriM82 may achieve a faster rate of grain filling when moisture availability is non-limiting (Fig. 3). Genotypic differences in the rate of grain filling have been reported for wheat (Gong *et al.* 2005) as well as differences induced by changes in moisture and nutrient status (Yang and Zhang 2006) and in a stay-green durum mutant (Spano *et al.* 2003).

Stay-green phenotype

The ability of a particular genotype to retain green leaf area for longer than a standard genotype during the grain-filling period

has been referred to as 'stay-green' phenotype (Thomas and Smart 1993). We observed that SeriM82 exhibited stay-green phenotype and higher yield than Hartog both in the presence and absence of moisture stress in all years and treatments where it exhibited a significant yield advantage. Further, SeriM82 failed to exhibit significantly higher yield than Hartog in 2006 when it was unable to express the stay-green phenotype. Thus, stay-green is associated with the high yield of SeriM82 in a range of moisture-stress environments typical of the deep Vertosol soils of the Australian northern grain belt. Stay-green is also known to be associated with improved yield and yield stability in sorghum in this region (Borrell *et al.* 2006). The stay-green phenotype has also been observed in SeriM82 in subtropical Mexico (Gutiérrez-Rodríguez *et al.* 2000; Reynolds *et al.* 2000b).

The physiological and genetic determinants of stay-green can vary among species and among genotypes within species (Silva *et al.* 2000, 2003; Thomas and Howarth 2000; Spano *et al.* 2003; Borrell *et al.* 2006; Rampino *et al.* 2006). Thomas and Howarth (2000) described 5 ways in which a functional stay-green phenotype may be generated. In plants such as *Lolium temulentum* it has been calculated that a delay of only 2 days in the onset of leaf senescence can result in an 11% increase in the carbon fixed by the plant (Thomas and Howarth 2000). Variation for stay-green has been reported for wheat but detailed studies concerning the underlying physiological mechanisms are currently limited (Silva *et al.* 2000, 2003; Spano *et al.* 2003; Rampino *et al.* 2006; Joshi *et al.* 2006). Joshi *et al.* (2006) reported that stay-green in spring wheat is under the control of around 4 additive genes and is associated with spot blot resistance. A stay-green durum mutant has been reported to have increased leaf area, and rate and duration of grain filling (Spano *et al.* 2003). Duration of green flag-leaf area was correlated with higher water-use efficiency during grain formation and considerable variation among a range of modern and older winter wheat genotypes was reported by Gorny and Garczynski (2002). Compared with wheat, understanding of the mechanisms underlying stay-green in sorghum is more advanced. In sorghum it has been proposed that higher green leaf area at anthesis, delayed onset of senescence, and a slower rate of senescence are the main components of stay-green and that there may be differences in the inheritance of the latter 2 (Thomas and Smart 1993; van Oosterom *et al.* 1996). Our data suggest that green leaf area at anthesis is not a major factor in the stay-green expressed by SeriM82, since SeriM82 exhibited higher leaf area at anthesis in only one tested environment (2004; Fig. 1) despite exhibiting higher yield and stay-green in 5 of the 6 environments (Table 2). For sorghum in Australia it has been shown that the importance of the timing of onset of senescence and rate of senescence vary among genotypes and can be affected by environment (Thomas and Howarth 2000; Borrell *et al.* 2006). The data for SeriM82 indicate that this stay-green wheat genotype may also exhibit variation with environment with respect to the rate of senescence and possibly the timing of onset. For the irrigated treatment in 2003 and rain-fed treatment of 2004 the timing of onset of senescence was similar for Hartog and SeriM82 but the rate of senescence was slower for SeriM82 (Fig. 2). In 2006 when

SeriM82 failed to exhibit significantly higher yield than Hartog it also failed to express stay-green phenotype (Table 2, Fig. 2).

SeriM82 had higher SLA in 2004 and 2005 than Hartog (Fig. 1). A lesser demand for carbohydrate to produce an equivalent leaf area could allow SeriM82 to divert more to other organs for root growth and grain filling. If SeriM82 is able to divert more resources to maintaining roots at depth late in the season this would improve its ability to continue moisture and nutrient uptake, as suggested in recent studies (Manschadi *et al.* 2006).

Nitrogen dynamics are clearly an important factor in the maintenance of green leaf area. For example, in certain stay-green sorghum hybrids, changes in the balance between N demand and supply during grain filling compared with senescent genotypes are thought to result in a slower rate of N translocation from the leaves to the grain thus helping to maintain green leaf area (Borrell and Hammer 2000). There are currently insufficient data to allow detailed characterisation of differences in N dynamics between SeriM82 and Hartog. However, data for a single season in 2004 do not suggest a higher specific leaf nitrogen content in leaves of SeriM82 at anthesis nor a slower rate of translocation from the leaves during grain filling (data not shown).

Water use and soil moisture extraction

The ability of SeriM82 to maintain green leaf area and to increase biomass later in the season during terminal drought, as in 2004 and the rain-excluded treatment of 2003, suggests that it has access to soil moisture later in the season. However, repeated-measurements over several contrasting seasons failed to detect a consistent difference in the depth of soil moisture extraction or in the total moisture extracted. Likely explanations for such a discrepancy would include that SeriM82 (1) has higher intrinsic transpiration efficiency (TE), or (2) gains access to more water later in the season by deferring water use from pre- to post-anthesis, or (3) extracts a greater amount of deep soil moisture but the amount is not detectable within the error of measurement. There is no evidence to suggest that the higher yield of SeriM82 is due to high TE, resulting from more conservative stomatal control as exhibited by the wheat line Quarrion, and now deployed in Australian wheat cultivars such as Drysdale and Rees (Rebetzke *et al.* 2002). Published stable carbon isotope discrimination values indicate that neither Hartog nor SeriM82 has this type of higher TE (Condon *et al.* 1990; Condon and Richards 1992; Fischer *et al.* 1998; Rebetzke *et al.* 2002). Another mechanism for achieving higher TE is through increased photosynthetic capacity (reviewed in Condon *et al.* 2002). We are not aware of any detailed measurements of the photosynthetic capacity of SeriM82 and Hartog in carefully controlled conditions. However, there is some evidence suggesting that higher TE in SeriM82 based on increased photosynthetic capacity is unlikely. First, assimilation measurements in the field did not provide evidence for higher photosynthetic capacity in SeriM82 under non-limiting moisture conditions in 2003 (data not shown). Second, measurements of leaf greenness, a surrogate for leaf chlorophyll content, do not suggest a higher maximum leaf chlorophyll concentration in

SeriM82 than in Hartog (Fig. 2). This then leaves the possibilities for differences in water availability late in the season due to either postponement of water use early in the season, or greater extraction at depth later in the season. There is some evidence for the hypothesis that SeriM82 can extract more soil moisture deep in the profile late in the season than can Hartog. Experiments where single plants were cultured in large root observation chambers have demonstrated that SeriM82 can extract more soil moisture per soil volume than Hartog, particularly at depth later in the season (Manschadi *et al.* 2006). Differences in the distribution patterns of the roots contributed to this difference in moisture extraction. In root-chamber experiments the roots of SeriM82 had more restricted lateral growth than those of Hartog but were more densely distributed, particularly deeper in the profile and late in the season. In addition, computer simulations indicated that such differences in root architecture and water extraction would lead to more water extraction during grain filling. Computer simulations of the marginal WUE of soil moisture extracted during the grain-filling period gave estimates ranging from 5.0 to 5.8 g grain/m².mm, which is much higher than WUE calculated for water extracted over the whole growing season (Manschadi *et al.* 2006). This agrees with other reports of high marginal WUE during the grain-filling period (Passioura 1972; Gorny and Garczynski 2002; Kirkegaard *et al.* 2007). Remarkably, for a marginal WUE figure of 5.5 g grain/m².mm the yield differences between SeriM82 and Hartog observed under conditions of terminal moisture stress, such as occurred in the rain-excluded treatment in 2003 (60 g/m²; Table 2) and in 2004 (70 g/m²), could be achieved by extra moisture extraction during the grain-filling period in the order of only 11 and 13 mm, respectively. Such small differences in extraction are difficult to detect in the field with current soil-moisture measurement technology and are within the experimental error that we observed (Fig. 5, Table 3). Further circumstantial evidence for the crucial role of water availability late in the season comes from data in 2006 where soil moisture was restricted to the top 0.70 m of the soil profile and where plants were heavily dependent on in-crop rainfall and irrigation. Under these conditions, SeriM82 did not exhibit stay-green phenotype nor a significant yield advantage over Hartog (Table 2, Fig. 2). Thus,

the evidence suggests that the availability of deep soil moisture late in the season is important for the expression of stay-green phenotype and higher yield in SeriM82. On the basis of current evidence we cannot rule out the possibility that there are small differences in water extraction pre- and post-anthesis and/or simply in the amount of water extracted deep in soil profile late in the season, which are sufficient to explain the observed yield differences but have not yet been detected experimentally in the field.

Our results indicate that differences in biomass, leaf area, and leaf greenness up to the time of anthesis are generally small and would not provide a practical means of selecting for the stay-green phenotype found in SeriM82. Any differences in soil moisture extraction are also hard to detect. Differences in green leaf area retention following anthesis appear to provide the best discrimination. It is known that the green leaves and particularly the flag leaf are major contributors of photosynthate to the developing grain and that green leaf area retention is associated with higher water-use efficiency of grain formation (Thorne 1982; Gorny and Garczynski 2002). Therefore, it has been proposed that selection for stay-green should be beneficial for wheat production under harsh environments (Reynolds *et al.* 1999). We found that SPAD measurements of flag leaf greenness provided a useful method for detecting the difference between genotypes. In addition it is plausible that differences in canopy temperature might also provide a means to screen for differences in soil water extraction (Reynolds *et al.* 2006).

The stay-green mechanism observed in SeriM82 leading to higher yields than in Hartog in a wide range of sites and seasons in the Australian northern grain belt would not be expected to offer the same level of advantage in all cropping environments. SeriM82 failed to express stay-green or higher yield in 2006 when deep soil moisture was lacking. This indicates that the advantage to SeriM82 may be less in environments where (a) soils are shallow and/or of low water-holding capacity and (b) crops rely primarily on in-season rainfall such as in Mediterranean environments. Thus, the stay-green mechanism exhibited by SeriM82 might not provide a significant yield advantage in cereal cropping areas in several of the cropping regions of the world, including large areas of the southern and western grain belts of Australia. This is consistent with the observation that the yield advantage of SeriM82 in MET in Australia was greater in the northern grain belt than in areas of Mediterranean climate (Sivapalan *et al.* 2001).

Table 3. Net soil moisture (mm) extraction from sowing to maturity of wheat genotypes SeriM82 and Hartog in the field at Kingsthorpe in subtropical Australia

Values represent the total initial minus final volumetric soil moisture content for the measured soil profile. PAWC to 1.5 m for 2003 and 2004, to 1.9 m for 2005, and to 0.7 m for 2006. Values for genotypes for each treatment in each year are not significantly different ($P > 0.05$)

Year	Treatment	Change in soil moisture		n
		Hartog	SeriM82	
2003	Irrigated	105 ± 11	107 ± 12	4
	Rain excluded ^A	94 ± 11	117 ± 13	4
2004	Rain-fed	156 ± 4	150 ± 6	3
2005	Rain-fed	151 ± 10	127 ± 4	3
2006	Profile depleted	77 ± 4	76 ± 7.1	3

^ARain excluded post anthesis at approximately 79 days after emergence for 2003 only.

Conclusions

SeriM82 achieved higher yield than Hartog through higher grain mass by maintaining green leaf area. Compared with Hartog, SeriM82 continued to accumulate biomass in the grain for longer during the grain-filling period when marginal WUE was high. The availability of deep soil moisture appears necessary for the expression of high yield and the stay-green phenotype. The exact mechanism by which SeriM82 maintains green leaf area later in the season in the absence of a detectable difference in soil moisture extraction in the field has yet to be fully elucidated. However, we consider the most likely explanation is that SeriM82 extracts a small amount of extra deep soil moisture

late in the season and/or can defer some water use from pre- to post-anthesis. Several shoot and root traits are involved. These are influenced to varying degrees by the environment. The failure of SeriM82 to express stay-green when deep soil moisture was unavailable suggests that the phenotype of SeriM82 is best suited to soils that have moisture available at depth. Such soils occur widely in the Australian northern grain belt. Different trait combinations will likely be needed in other cropping environments.

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