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Associations of *NAM-A1* alleles with the onset of senescence and nitrogen use efficiency under Western Australian conditions

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Abstract Wheat grain yield and protein content are significantly influenced by the onset of senescence and the duration of the grain filling phase. The onset of senescence also affects Nitrogen use efficiency (NUE) through interacting pathways involving N accumulation and translocation of N into the grains. The objective of this study was to relate variation in NUE and its components with two groups of the *NAM-A1* gene alleles; (i) early onset of senescence in cultivars carrying the *NAM-A1a* allele, (ii) delayed onset of senescence in cultivars carrying the Non-*NAM-A1a* allele (*b*, *c*, *d*) in wheat cultivars grown under Western Australia conditions. A field trial was carried out over two seasons examining 19 cultivars under different N rates and time of N application. The Normalized Difference Vegetation Index was utilized to determine the onset of senescence after anthesis. The early onset of senescence results in high grain yield, harvest index, and NUE due to improvements in the N utilization ability. Accelerating the onset of senescence results in a short grain filling period leading to grain maturity before the onset of unfavourable summer conditions. The function of alleles of *NAM-A1* gene in controlling senescence hence the NUE is highly regulated by environmental conditions. This study concluded that the function of *NAM-A1a* allele induces the onset of senescence with a positive effect on the NUE and its components under Western Australian conditions.

Keywords *NAM-A1* gene · Nitrogen use efficiency (NUE) · Normalized difference vegetation index · The onset of senescence

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Introduction

Nitrogen use efficiency (NUE) of cereal crop production is around 33% due to the loss of N fertilizer by soil

system and the low efficiency of N element uptake and usage by the crop (Raun and Johnson 1999). NUE can be improved by optimising N fertilizing regime and selecting cultivars with a better N uptake and utilization efficiency. Nitrogen uptake efficiency (NUpE) indicates the capacity of the plant to adsorb N from the soil, while nitrogen utilization efficiency (NUtE) indicates the amount of grain production by available N in the plant (Hirel et al. 2007; Lea and Azevedo 2006). Moreover, N utilization efficiency (NUtE) includes both N assimilation efficiency (NAE) and N remobilization efficiency (NRE) (Avice and Etienne 2014). Many studies have found that NUpE is a key contributor to NUE at all N conditions (Dhugga and Waines 1989; Hitz et al. 2017), while NUtE is considered more as genetic variation of NUE, most influential under low nitrogen conditions (Le Gouis et al. 2000; Wang et al. 2011). On the other hand, Barraclough et al. (2010) found that NUtE explained most of the variations in grain yield than NUpE under low and high N condition (Barraclough et al. 2010). This view is supported by Gaju et al. (2011) who reported that genetic variation in NUE under low N is more correlated to the diversity of NUtE compared to that of NUpE (Gaju et al. 2011).

Grain filling usually depends on the amount of N remobilized during grain filling from the accumulated N in the plant during the vegetative phase (Barbottin et al. 2005; Palta et al. 1994). Thus, N stored at pre-anthesis is crucial in wheat particularly in the Mediterranean climate like Western Australia due to the relatively shorter grain filling period. Many studies have shown that about 60–95% of grain N come from the remobilization of N stored in plant parts before anthesis (Barraclough et al. 2010; Hirel et al. 2007; Kong et al. 2016). Therefore, a better understanding of the mechanisms affecting NUtE could improve NUE of crops through breeding programs. Senescence is the final developmental stage of plant cells allowing recycling of nutrients from vegetative parts to the developing grains which is strongly influenced by both the genetic and environmental factors (Distelfeld et al. 2014; Lim et al. 2007). Nitrogen availability has a strong effect on senescence timing as senescence relies on the balance between N availability and the N demand by the plant (Triboi and Triboi-Blondel 2002). For instance, low N conditions lead to accelerated senescence, while high N conditions can delay senescence (Gan and Amasino 1997; Martre et al. 2006). In contrast, genetic factors modulate this balance either by a direct effect on the key factors of the senescence such as N remobilization and uptake or through an indirect effect on the ratio between source and sink organs (Bogard et al. 2011). In wheat, the onset of senescence is correlated with the translocation of nutrients from leaves to developing grains which influences the utilization efficiency. Both the C and N accumulation and C and N translocation into the grains are involved in maintenance of green leaves and the onset of senescence (Kipp et al. 2014). The functional *NAM-B1* gene, which encodes for a transcription factor of the NAC family, accelerates senescence and increases nutrient remobilization from leaves tissue to the grains (Waters et al. 2009).

Senescence can influence crop production in two ways, by modifying the nutrient remobilization efficiency, or by changing the duration of photosynthesis. The *Gpc-B1* or *NAM-B1* gene from wild emmer wheat is reported to be a genetic factor to improve grain protein content in bread wheat without reducing grain yield (Eagles et al. 2014). However, some controversial results have been reported towards its effects on grain yield. Asplund et al. (2010) reported a negative yield effect from functional *NAM-B1* allele. Most of the bread wheat cultivars worldwide carry a non-functional allele of *NAM-B1* (Hagenblad et al. 2012). Its homeolog *NAM-A1* is a gene with similar function that locates on chromosome 6A and is also associated with nutrient remobilization to the grain and accelerated senescence (Cormier et al. 2015). So far there are four different *NAM-A1* alleles detected in bread wheat cultivars in Australia and world wide (Cormier et al. 2015; Yang et al. 2018). The *NAM-A1a* allele is mainly found in spring wheat cultivars with high grain protein content (GPC), low grain yield and short growing season. The *NAM-A1d* allele is mostly represented in modern European cultivars with a high grain yield. Cultivars carrying the *NAM-A1b* and *c* alleles were intermediate between those carrying *NAM-A1a* and *NAM-A1d*.

Many studies are arguing that the stay-green cultivars with a slower senescence rate possess longer grain filling period through continued N uptake and translocation (Barraclough et al. 2010; Hitz et al. 2017; Kipp et al. 2014). Stay-green cultivars with greater N uptake, accumulation, and translocation capabilities deliver extra metabolic gains in NUE (Christopher et al. 2008). Delayed leaf senescence also

Table 1 Heading date and maturity for 19 Australian wheat cultivars carrying a different allele of the *NAM-A1* gene

Group	*NAM-A1*	Heading date	Maturity	Cultivar
NAM-A1 a	a	105	Early–mid	Baxter
	a	103	Early	Bonnie-Rock
	a	109	Early–mid	Chara
	a	102	Early	H45
	a	100	Early	Livingston
	a	101	Early	Spitfire
	a	101	Early–mid	Westonia
	a	104	Early–mid	Wyalkatchem
Non-NAM-A1 a	c	104	Mid	Alsen
	c	101	Early	Drysdale
	b	107	Early–mid	Excalibur
	c/d	108	Mid	Gladius
	c	110	Mid–long	Gregory
	a/c	105	Early	Kukri
	d	108	Early–mid	Mace
	c	109	Early–mid	Pastor
	c	103	Mid–long	RAC875
	c	101	Early	Volcani
	d	112	Mid–long	Yitpi

provides additional carbon and nitrogen to be allocated to the roots of stay-green cultivars during grain filling that increases the capacity to extract N from the soil compared to shorter grain filling cultivars (Borrell et al. 2001; Martre et al. 2007). In summary, yield and grain protein content are strongly influenced by the duration of the post-anthesis phase and the stay green traits. These findings enhance our understanding in regard to the onset and rate of senescence after anthesis that also influence NUE and its components. The objective of this study is to characterize the influence of *NAM-A1* alleles on the senescence and stay-green of Australian wheat cultivars and establish a relationship between the presence or absence of high grain protein content associated *NAM-A1a* allele with grain yield and NUE components in Western Australian conditions.

Materials and methods

Experimental treatments

Field trials were carried out at two sites in Western Australia. The first trial was conducted at Broomehill (Katanning, Western Australia) in 2015 and the second trial was conducted at the Agriculture and Food of Western Australia research station at Wongan Hills, Western Australia in 2016. Information about the monthly rainfall, the minimum temperature and the maximum temperature of these sites is given in Supplementary Table 1. The same experimental regimens were tested at both sites on 19 cultivars (Table 1). All cultivars are nominations from Australian breeding programs to represent the genetic diversity amongst Australian wheat cultivars (Bioplatforms 2016). The cultivars were classified into two groups (Table 1) based on the different alleles of *NAM-A1* (Yang et al. 2018). Additionally, *NAM-A1* allele information and maturity type of further 31 cultivars involved in the Australian National Variety Trial (NVT) has been collected (Table 2, Yang et al. 2018; https://www.nvtonline.com.au). In each experiment we used a split-plot design, in which cultivars were randomized on main plots, and N treatments were randomized on the sub-plots, and each treatment was replicated three times. The sub-plot size was 3 m × 1.25 m with a 0.5 m gap between the sub-plots. The sowing dates were 17 June in 2015 and 16 May in 2016, which are the recommended dates for wheat in these agricultural regions of Western Australia. The nitrogen treatment included three levels:

Table 2 *NAM-A1* allelic characteristics and maturity type information of additional 31 Australian cultivars

Maturity	NAM-A1 allele	Allele percentage	Cultivar
Early	a	100% NAM-A1 a	B53
Early	a		Tenfour
Early	a		AC Barrie
Early	a		Lincoln
Early	a		Merlin
Early–mid	d	80% NAM-A1 a 20% Non-NAM-A1 a	Cobalt
Early–mid	a		Cobra
Early–mid	a		Corack
Early–mid	a		Cosmic
Early–mid	a		Emu Rock
Early–mid	a		Hydra
Early–mid	a		Impress CL plus
Early–mid	d		Jade
Early–mid	a		Superme
Early–mid	a		Wallup
Mid	d	63% Non-NAM-A1 a 37% NAM-A1 a	Beckom
Mid	d		Bremer
Mid	c		Eagle Rock
Mid	d		Grenade CL plus
Mid	a		Lancer
Mid	a		Magenta
Mid	d		Scepter
Mid	a		Suntop
Mid–long	c	100% Non-NAM-A1 a	Brindawarra
Mid–long	c		Calingiri
Mid–long	d		Cutlass
Mid–long	d		Scout
Mid–long	d		Trojan
Mid–long	d		Tungsten
Mid–long	c		Zen

low N (LN) 0 kg N ha^{-1}, mid N (MN) 50 kg N ha^{-1} and high N (HN) 100 kg N ha^{-1}, plus 20 kg N ha^{-1} as a basal rate applied at sowing. The timing of the N application was synchronised to several Zadoks (Z) growth stages of the cultivar (Zadoks et al. 1974). Accordingly, these were: T1 = 100% of N rate was applied at mid-tillering (Z22–Z24); T2 = 100% of N rate was applied at booting (Z43–Z45); and T3 = 50% of N rate was applied at mid-tillering and 50% of N rate was applied at booting. Flexi-N (42.2% of N) was applied as a source of N for the foliar applications and includes three types of N: 50% urea, 25% nitrate and 25% ammonium. The nitrate is available directly to the plants while the urea and ammonium are less soluble enabling a controlled release of nitrogen over an extended period (CSBP 2012).

Yield components

Anthesis date was recorded in each plot for each cultivar when 50% of the heads carried visible stamens (Zadoks growth scale 61). Grain and straw samples were harvested on the 23rd of November in 2015 and on the 8th of November in 2016 when all plants were completely mature by visual inspection. All plants in each plot (main stem plus tillers) were harvested to measure the yield components. Before mechanical harvesting, a quadrat of 0.44 m^2 of plant material was

cut off at ground level within each plot using a small hand harvester. All samples were then oven dried separately in a forced air circulating dryer at 60 °C for 72 h. Grain and straw yield was estimated, and the grain protein content and residual N in straw were analyzed using a FOSS XPS Near-infrared reflectance (NIR) equipment with a model 5000 spinning cup. NIR data analysis was collected using WinISI software (FOSS NIR Systems Inc., Laurel, MD, USA). The residual N concentration in straw was calculated using both free nitrogen and protein/amino-acid bound divided by 4.43 (Yeoh and Wee 1994). Grain weight and size were estimated on a representative grain sample of 20 g. The number of grain head^{-1} was estimated by counting the number of grains per three heads using a seed counter. Harvest index (HI) and N harvest index (NHI) were obtained by calculating the ratio of grain or N at harvest to total above-ground biomass or N, respectively (Gaju et al. 2011; Siddique et al. 1989). N uptake efficiency (NUpE) was calculated as the ratio of above-ground N uptake to total N supply. N utilization efficiency (NUtE) was calculated as the ratio of grain yield to above-ground N uptake. N use efficiency (NUE) was calculated as the ratio of grain yield to total N supply or multiplying the NUpE by NUtE (Moll et al. 1982).

Normalized difference vegetation index

The GreenSeeker Hand Held Sensor (Trimble Navigation, Sunnyvale, CA) was used to measure the normalized difference vegetation index (NDVI). The NDVI measurement was recorded at seven growth stages [stem elongation (Z32–Z35), booting (Z43–Z45), anthesis (Z61), 10 days after anthesis (DAA), 20 DAA, 30 DAA, and 40 DAA] (Zadoks et al. 1974). The NDVI values were calculated as (ρNIR–ρRed)/(ρNIR + ρRed), where ρNIR and ρRed are respectively the fractions of emitted near infrared (NIR) and red radiation reflected back from the sensed area (Macnack et al. 2014).

The onset and rate of senescence in the whole plant canopy were determined when the chlorophyll content of the plant rapidly decreases during grain filling (Araus and Labrana 1991). Averaged across all cultivars, the maximum NDVI was achieved at anthesis stage (Zadoks growth scale 61). The onset of senescence was determined when the maximum NDVI decreased by 10% after anthesis of each cultivar (Christopher et al. 2014). Rate of senescence was calculated as $(NDVI_{Max} - NDVI_X)/NDVI_{Max} \times 100$, where $NDVI_{Max}$ is the value of maximum NDVI of the plant before decreases during grain filling and $NDVI_X$ is the value of NDVI in the specific stage to measure the decline of rate senescence.

Statistical analysis

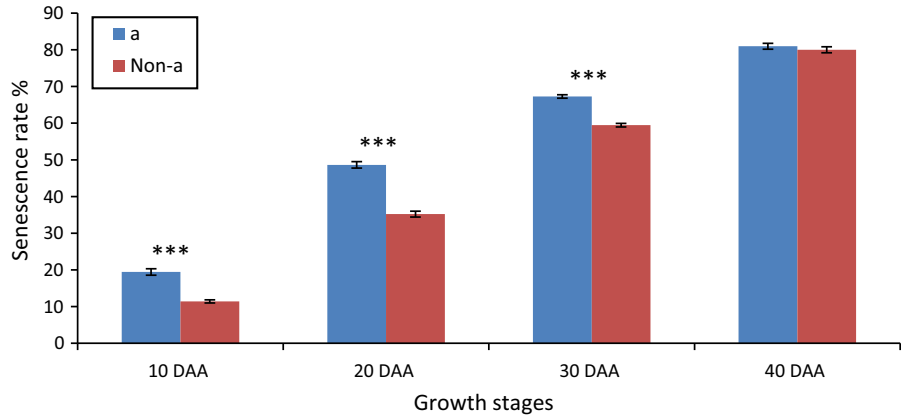
An analysis of variance (ANOVA) was performed using the Genstat software (VSNi Pty Ltd, UK) after the initial evaluation of effects at the site to determine genotype, nitrogen treatment effects at different time application. This split-plot trial-design structure was incorporated into the analysis. In the case of significant differences based on ANOVA and F-values for treatment effects, LSD ($p < 0.05$) test and standard deviation/error of means were used to identify significant means. Correlation analysis was conducted to investigate the relationship between *NAM-A1a* and Non-*NAM-A1a* genotypes and NUE and its component. Comparisons between groups of correlation were undertaken by converting to z-scores and comparing significance. In situations where there was not significant different between years when taking into account the treatment effects, then the data were combined for further analysis.

Results

The recorded climate data of daily temperature and rainfall demonstrated a stable growth conditions during the season without any occurrence of major drought or heat stress periods in both experiments. The rainfall was higher in 2016 than in 2015 particularly during the tillering and stem elongation stages (i.e. sowing date Z0 to mid booting Z45). The temperatures were similar in the two seasons when daily mean temperatures were on average 1.5 °C lower in 2015 (Supplementary Table 1).

Identification of early senescence and stay-green cultivars

The identification of early senescence and the stay-green cultivars was based on the NDVI data after anthesis. Eight cultivars belong to early senescence



*** Significant at the 0.001.

Fig. 1 The onset and rate of senescence of whole plant at four stages (10 DAA, 20 DAA, 30 DAA, 40 DAA) for two groups (*NAM-A1a* and Non-*NAM-A1a*) of wheat cultivars

Table 3 Effects of different *NAM-A1* alleles, N rates, and time of N application on heading date, above-ground biomass (AGB), number of seed head^{-1}, grain thousand weight (GTW), residual N in straw (RNS), grain protein content (GPC)

Groups	Heading Day	AGB kg ha^{-1}	No. Seed Head^{-1}	TGW g	RNS %	GPC %
NAM-A1 a	103.9 b	5254.0	33.02	37.4 b	2.20 b	12.4 b
Non-*NAM-A1 a*	106.0 a	5179.5	33.27	38.1 a	2.23 a	12.7 a
N rates						
0	104.8	4606.4 b	31.27 b	39.5 a	2.13 c	11.1 c
50	104.8	5572.6 a	34.07 a	36.9 b	2.24 b	12.8 b
100	105.0	5635.1 a	34.12 a	36.8 b	2.29 a	13.8 a
Time N app						
T1	104.9	5436.1 a	32.97	38.4 a	2.19 b	12.3 c
T2	104.8	5259.9 b	33.34	37.9 a	2.24 a	12.4 b
T3	104.9	5118.0 c	33.15	36.9 b	2.22 a	12.8 a

Within the columns in each factor, means followed by the same letter are not significantly different according to LSD ($p = 0.05$). T1 = 100% of N rate was applied at mid-tillering (Z22–Z24); T2 = 100% of N rate was applied at booting (Z43–Z45); and T3 = 50% of N rate was applied at mid-tillering and 50% of N rate was applied at booting

group, and 11 cultivars belong to stay green group. The onset of senescence showed a wide variation among the two groups (*NAM-A1a* and Non-*NAM-A1a*). Early onset senescence was found in cultivars carrying the *NAM-A1a* allele; however, in cultivars carrying the Non-*NAM-A1a* allele the onset of senescence was delayed especially for these carrying alleles *c* and *d*. As shown in Fig. 1, the *NAM-A1a* group reached to the 50% of senescence about 20 DAA, almost 7 days earlier than Non-*NAM-A1a* group. When averaged over the years and N treatments, the mean heading date of *NAM-A1a* group was 103.9 days after sowing and the Non-*NAM-A1a* group was 106 days after sowing (Table 3). To further confirm, the information of the allelic composition of *NAM-A1* and maturity type of additional 31 cultivars (Table 2) have been collected (Yang et al. 2018; https://www.nvtonline.com.au). In general, presence of *NAM-A1 a* allele was enriched in early and early to mid maturity type cultivars while *NAM-A1 c* and *d* alleles were more characteristic at mid and mid to long maturity type cultivars. Early maturity type cultivars exclusively possess *NAM-A1 a* allele and 80% of the early to mid maturity type cultivars carry the same allele. On the

Table 4 Effects of different *NAM-A1* allele, N rates, and time of N application on grain yield (GY), harvest index (HI), N harvest index (NHI), N utilization efficiency (NUtE), N uptake efficiency (NUpE), and N use efficiency (NUE)

Groups	GY kg ha^{-1}	HI %	NHI %	NUtE kg grain kg N ha^{-1}	NUpE kg N ha^{-1} kg N supply^{-1}	NUE kg grain kg N supply^{-1}
NAM-A1 a	2200.8 a	0.42 a	0.80 a	3.9 a	12.2	49.9 a
Non-*NAM-A1 a*	2062.1 b	0.40 b	0.78 b	3.7 b	12.0	44.6 b
N rates						
0	1879.6 b	0.41	0.78 b	4.1 c	22.1 a	90.7 a
50	2266.4 a	0.41	0.80 a	3.7 b	8.7 b	32.5 b
100	2259.3 a	0.40	0.80 a	3.5 c	5.4 c	18.9 c
Time N app						
T1	2186.6	0.41	0.79	3.8	12.1	47.6
T2	2130.7	0.41	0.79	3.8	12.1	47.4
T3	2087.9	0.41	0.79	3.7	12.1	47.1

Within the columns in each factor, means that show significant differences are labelled with different letters according to LSD ($p = 0.05$). T1 = 100% of N rate was applied at mid-tillering (Z22–Z24); T2 = 100% of N rate was applied at booting (Z43–Z45); and T3 = 50% of N rate was applied at mid-tillering and 50% of N rate was applied at booting

other hand, 70% of mid maturity type cultivars have *NAM-A1 c* or *d* alleles (Non-*NAM-A1 a* group). Moreover, all the mid to long maturity type cultivars possess *NAM-A1 c* or *d* alleles.

Grain yield, yield components, aboveground biomass and HI

There was a significant difference ($p < 0.001$) in grain yield among the two groups and in response to N rate and time of N application (Table 4). Averaging across the N rates and time of application, cultivars of *NAM-A1a* group produced significantly higher grain yield (2200.8 kg ha^{-1}) than the Non-*NAM-A1a* group (2062.1 kg ha^{-1}). The maximum yield was achieved with 50 and 100 kg N ha^{-1}; while the lowest yield was obtained from the control treatment (0 kg N ha^{-1}). The timing of N application also influenced the grain yield. The highest grain yield (2186.6 kg ha^{-1}) was achieved by applying N at the early stage (Mid-tillering) while applying N at the late stage (Booting) produced a lowest grain yield (2087.9 kg ha^{-1}). Cultivars group of Non-*NAM-A1a* produced the higher thousand grain weight (TGW) (38.1 g), while cultivars group of *NAM-A1a* produced (37.4 g). In the case of N rates, the control (0 kg N ha^{-1}) achieved the highest GTW (39.6 g). On the other hand, applying N at mid-tillering produced the greatest GTW (38.4 g) while applying the N at the late stage (booting) provided the lowest weight (37 g). The above-ground biomass was significantly affected among the two groups, N rates and the time of N application ($p < 0.001$). The above-ground biomass was increased with increasing applications of N, and the highest was at 100 kg N ha^{-1}. The timing of the N application contributes to the above-ground biomass; the maximum weight was achieved when N was applied at mid-tillering stage (5436.1 kg ha^{-1}). N rates influenced the numbers of grain head^{-1} when 100 kg ha^{-1} N produced the highest numbers of grain head^{-1} (34.12 g). Furthermore, cultivars of the *NAM-A1a* group had a significant higher HI 0.42% compared to the Non-*NAM-A1a* group of 0.40% ($p < 0.001$).

Grain protein content, residual N in straw, and Nitrogen Harvest Index

Grain protein content and straw nitrogen content were significantly different ($p < 0.001$) among the cultivars, the N rate, and the time of N application. Cultivars not possessing the *NAM-A1a* allele had higher grain protein content and higher residual N in the straw (Table 3). However, cultivars in the *NAM-A1a* group had a higher NHI (0.80) than cultivars in the Non-*NAM-A1a* group (0.78). Across all the N rates, the grain protein content, residual N in straw, and NHI, the highest values were achieved by the 100 kg N ha^{-1} treatment. Late stage N application

Table 5 Correlation between the rate of senescence (Sen) at 10, 20, 30, 40 days after anthesis (DAA) with NUE and agronomic traits of two groups (*NAM-A1a* and *Non-NAM-A1a*) of Australian wheat cultivars under different N conditions and time of applications

	NAM-A1a group				Non-*NAM-A1a* group			
	Sen 10%	Sen 20%	Sen 30%	Sen 40%	Sen 10%	Sen 20%	Sen 30%	Sen 40%
Heading	− 0.35	− 0.41	− 0. 51	− 0.64	0.29	0.31	0.29	0.24
AGB	0.85**	0.84**	0.82**	0.73*	0.88**	0.90**	0.88**	0.81**
No. seed	0.62	0.75*	0.72*	0.64	0.42	0.45	0.41	0.32
TGW	− 0.15	− 0.31	− 0.24	− 0.11	− 0.61	− 0.64	− 0.58	− 0.39
RNS	0.23	0.36	0.29	0.16	0.70*	0.73*	0.66	0.44
GPC	0.42	0.51	0.43	0.26	0.77*	0.80**	0.73*	0.51
GY	0.82**	0.89**	0.87**	0.78*	0.81**	0.82**	0.81**	0.77*
HI%	− 0.60	− 0.49	− 0.46	− 0.35	− 0.73*	− 0.74*	− 0.68*	− 0.45
NHI%	0.43	0.56	0.50	0.35	0.80**	0.82**	0.78*	0.63
NUtE	− 0.50	− 0.59	− 0.52	− 0.35	− 0.81**	− 0.84**	− 0.77*	− 0.56
NUpE	− 0.69*	− 0.77*	− 0.71*	− 0.56	− 0.89**	− 0.90***	− 0.85**	− 0.66
NUE	− 0.64	− 0.77*	− 0.64	− 0.56	− 0.89**	− 0.90***	− 0.85**	− 0.66

*, **, ***Significant at the 0.05, 0.01and 0.001 probability level, respectively. Senescence at 10 days after anthesis (Sen 10 DAA), senescence at 20 days after anthesis (Sen 20 DAA), senescence at 30 days after anthesis (Sen 30 DAA), senescence at 40 days after anthesis (Sen 40 DAA), heading date, above-ground biomass (AGB), number of seed/head, thousand grain weight (TGW), residual N in straw (RNS), grain protein content (GPC), grain yield (GY), harvest index (HI%), N harvest index (NHI%), N utilzation efficiency (NUtE), N uptake efficiency (NUpE) and N use efficiency (NUE)

increased the grain protein content and residual N in the straw.

Nitrogen use efficiency and its components

NUE and its components NUtE and NUpE significantly differed ($p < 0.001$) amongst the cultivars and N rates (Table 4). Cultivars in the *NAM-A1a* group resulted in a significantly higher NUtE, NUpE and NUE values. The low treatment with N rate of 0 N kg ha^{-1} produced the maximum NUtE, NUpE, and NUE compared to the 50 and 100 kg N ha^{-1}.

Correlation between rate of senescence and agronomic traits

The association between the rate of senescence and NUE and its components and agronomic traits of the two groups (*NAM-A1a* and Non-*NAM-A1a*) of Australian wheat cultivars under different N conditions and time applications are shown in Table 5. The correlation analysis showed that there is a significant positive correlation between the rate of senescence measured at 10, 20, 30 and 40 DAA and above-ground biomass (r = 0.85, 0.84, 0.82, 0.73 respectively) within the *NAM-A1a* group, and (r = 0.88, 0.90, 0.88, 0.81 respectively) within the Non-*NAM-A1a* group. In general, the rate of senescence had a higher positive association with RNS, GPC and NHI, while we found a higher negative correlation with TGW and HI within the Non-*NAM-A1a* group than the *NAM-A1a* group. Moreover, there was a strong significant negative correlation between the rate of senescence at 10, 20 and 30 DAA and NUtE and NUE under Non-*NAM-A1a* group only (Table 5).

Discussion

A number of studies have been reported on the genetic and functional characterization of the *NAM-B1* gene (Asplund et al. 2013; Uauy et al. 2006b; Waters et al. 2009). However, there is no investigation on the allelic variation of *NAM-A1* gene and its effect on senescence under Mediterranean climate. Under Western Australia conditions (Mediterranean climate), wheat is grown when rainfall and temperature are favourable during the vegetative phase (i.e. May–September), but

with frequent heat and drought stress during grain filling phase (i.e. October–November). Therefore, the objective of the present study was to relate the impact of *NAM-A1* allelic variation to NUE and its component under Western Australia conditions by understanding its effect on the rate of senescence. The 19 cultivars used in the study are the nominations for Australian breeding programs to represent the genetic diversity of *NAM-A1* gene and contrasting responses to nutrient remobilisation amongst Australian wheat cultivars (Yang et al. 2018).

The correlation between the onset of senescence and *NAM-A1* allelic variation

The results allow us to better understand the associations between the allelic variation of *NAM-A1* alleles and the onset of senescence in relation to grain yield and NUE in Western Australian conditions. Age-related senescence occurs in the late developmental stage of the wheat plant cycle in which chlorophyll and proteins are recycled to complete grain formation. Araus and Labrana (1991) reported that the onset of senescence occurs when the chlorophyll content of the plant decreases rapidly after anthesis. Several studies utilized the Green-Seeker sensor (NDVI) to measure the total greenness of wheat (Babar et al. 2006; Hansen and Schjoerring 2003; Lopes and Reynolds 2012). Our results clearly show that the rate of senescence from anthesis (Fig. 1) is different for the two *NAM-A1* allelic groups, with earlier and faster onset of senescence in the *NAM-A1a* group and delayed onset of senescence within the Non-*NAM-A1a* group. As shown in Tables 1 and 2, cultivars that carry the *NAM-A1a* allele were exclusively characteristic of early and early to mid maturity types. However, genotypes with Non-*NAM-A1a* alleles especially alleles *c* and *d* were primarily characteristic on mid and mid to long maturity types. Our findings suggest that the *NAM-A1a* accelerates senescence compared to the Non-*NAM-A1a* allelic group. Earlier studies reported that the functional *NAM-B1* gene could accelerate senescence and increase nutrient remobilization compared to the non-functional *NAM-B1* allele that shows delayed onset of senescence (Asplund et al. 2013; Brevis and Dubcovsky 2010; Uauy et al. 2006a; Waters et al. 2009). Cormier et al. (2015) identified that the *NAM-A1* gene has the same role as *NAM-B1* gene associated with nutrient remobilization and senescence kinetics. Although they have only found a low frequency of the *NAM-A1a* allele characteristic on elite germplasm selected for grain yield it was more frequent in Nepalese genotypes cultivated within a short growing season. The current study shows that all of the early and 80% of the early to mid ripening cultivars possess the *NAM-A1a* allele and this allele was not found in any of the late ripening cultivars. These results confirm that higher frequency of *NAM-A1a* allele in early maturing genotypes is associated with short grain filling which is often characteristic on the growing seasons such as the Mediterranian Western Australian conditions.

Genetic variation of NUE and its components based on the *NAM-A1* gene

NUE is a complex trait that results from an interaction of various component traits such as HI%, NHI, NUtE and NUpE. The main factors involved in the uptake and utilization of nutrients resulting in differences in morphological, physiological and biochemical processes are also affected by NUE and its components. Hence, for improvements of NUE in wheat crops, it is essential to recover more N from the soil and fertilizers (better NUpE) and produce higher grain yields from the available N in the plant (better NUtE). Our results show that above-ground biomass positively correlates with grain yield ($r = 0.94$ and 0.98) and negatively with NUE ($r = -0.74$ and -0.82, Supplementary Tables 2 and 3) both in the *NAM-A1a* and Non-*NAM-A1a* groups, respectively. These findings suggest that cultivars with high above-ground biomass produce higher grain yield when N is not in a limiting condition (Gaju et al. 2016). This is a consequence of genetic mechanisms that contribute to high above-ground biomass production that also leads to enhanced N uptake under high N conditions and increased N storage capacity in the plant. This result is supported by the findings of Kamiji et al. (2014) showing a significant association between shoot N uptake and shoot biomass if high N supply is available. The *NAM-A1a* group produced a higher grain yield of 2200.8 kg ha^{-1}, while Non-*NAM-A1a* group had a lower grain yield of 2062.1 kg ha^{-1}. These indicated that the wheat cultivars with *NAM-A1a* allele are more efficient in conditions with limited rainfall and high temperature during the grain filling period (Supplementary Table 1), although experiments using

controlled genetic background (NILs, RILs or DH populations) or unstructured populations can be used to reveal these mechanisms.

In a Mediterranean climate such as Western Australia, wheat is grown when rainfall and temperature are favourable during the vegetative phase but with insufficient rainfall and higher temperatures at grain filling (i.e. October–November). Breeding wheat that delays the onset of senescence might enable a greater capacity for plants to accumulate more N during grain filling (Borrell et al. 2001). However, water deficit during grain filling will reduce photosynthesis activity and shorten the grain filling period (Tahir and Nakata 2005; Yang et al. 2000).

Early onset of senescence is a useful strategy for Western Australian conditions

Water deficit negatively affects the N uptake during grain filling and the remobilization of stored N into the grain of cultivars with Non-*NAM-A1*a alleles. On the other hand, accelerated senescence results in a shorter grain filling period and helps to avoid some of the unfavourable seasonal conditions, such as high temperature and low rainfall rate. This is characteristic on wheat cultivars with the *NAM-A1a* allele (early onset of senescence). Many studies reported that delay senescence had been always beneficial to yield under optimal growing conditions (Christopher et al. 2014; Derkx et al. 2012; Spano et al. 2003). In contrast, many studies argue that delayed senescence may be of no consequence under stress conditions, especially during grain filling phase (Blum 1997; Yang et al. 2000). This result is also confirmed by the differences in HI%, NHI%, NUtE and the residual N in above-ground biomass between two groups. The *Non-NAM-A1a* group had lower HI, NHI, NUtE and more residual nitrogen in the straw due to delay in the onset of senescence under stress conditions such as the Western Australia climate. Moreover, the results of the correlation analysis (Table 5) show the rate of senescence has a stronger correlation with NUpE, NUtE and NUE under Non-*NAM-A1a* group than *NAM-A1a* group. Generally, grain filling in wheat is supplied by two major sources: the current photosynthesis during grain filling phase and the contribution of stored assimilates in the plant parts before flowering (Plaut et al. 2004; Ehdaie et al. 2008; Maydup et al. 2010). However, the current assimilates produced by photosynthesis might be limited due to the decline of leaf stomatal conductance under stress conditions (Blum 1997; Wang et al. 2015). Accordingly, the contribution of stored assimilates before flowering could be the principal source into the developing grains. According to Palta et al. (1994) the participation of current post-anthesis assimilation decreased by 57%, while the remobilization of stored assimilates increased by 36% into the total grain under water stress conditions. Taken together, *NAM-A1a* allele contributes to the onset of senescence and allows more effective translocation of nutrients to the grain, especially under stress conditions. Similarly, a negative correlation between the early onset of senescence and grain yield was by (Derkx et al. 2012; Jiang et al. 2004; Kichey et al. 2007; Kipp et al. 2014). However, many studies have found a positive relationship between stay-green phenotypes and grain yield (Borrell et al. 2001; Christopher et al. 2008; Gaju et al. 2016). As discussed by Bogard et al. (2011) the effect of the stay-green phenotype on the grain yield and NUE strongly depends on the environmental conditions that also include the available nutrients. Based on the current results, we can conclude that the presence of *NAM-A1a* allele accelerates senescence while the other alleles (*b*, *c*, *d*) delay the onset of senescence in the Mediterranean conditions that are often characterized by the dry season finish. Therefore, the presence of *NAM-A1a* allele can improve the nitrogen utilization by shortening the grain filling period leading to the ripe grain before the unfavourable seasonal conditions occur in Western Australia. Taken together, our study clearly demonstrates that the *NAM-A1a* allele is a desirable allele for Western Australian conditions where during the later grain filling stages plants do not have adequate water available to maintain a regular N remobilization. The *NAM-A1a* allele with its contribution to the enhanced N remobilization ability together with the early onset of senescence makes the wheat grown at Mediterranean climate condition achieve the maximum yield potential.

Conclusion

The *NAM-A1a* allele facilitates the onset of senescence, while the other alleles have a negative impact on the onset of senescence. In the Mediterranean

climate such as the Western Australian growing seasons with a dry finish, wheat with a shortened grain filling will benefit from the presence of the *NAM-A1a* allele. Accelerating the onset of senescence results in a shorter grain filling period leading to faster grain maturation to avoid the unfavourable seasonal conditions. Water deficit and high temperatures during the growing seasons in wheat growing regions are likely to become more prevalent due to the climate change, making it necessary to understand the genetic diversity of the wheat genotype basis of maturity to avoid the unfavourable seasonal conditions.

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