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REGULAR ARTICLE

Natural variation in the regulation of leaf senescence and relation to N and root traits in wheat

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Abstract
Objectives To identify parameters that can be used for the analysis of natural variation in leaf senescence of wheat; and to understand the association between the onset and progression of leaf senescence with N uptake and root traits.
Methods Chlorophyll content and the proportion of yellow leaves were used as senescence indicators and their relation with other morphological and physiological traits were measured in contrasting early senescing (ES) and late senescing (LS) wheat lines.
Results There were significant genotype effects on the onset and progress of senescence. The ES lines in which leaf senescence commenced early had significantly lower root biomass and N uptake than LS lines. The strong negative association between the extent of leaf senescence with root biomass and N uptake indicated that the poor root growth induced N limitation caused the early senescence of ES lines.
Conclusions The leaf senescence development in ES lines was precocious and constitutive as the trait expressed even under optimal growth conditions suggesting they could be useful in understanding the genetic regulation of senescence under different abiotic stress situations. Accelerated leaf senescence in wheat could be a mechanism to compensate for limitations in the root system that tend to restrict nutrient uptake.

Keywords Wheat · Senescence · Root traits · N uptake · N uptake efficiency

Introduction

Senescence plays a vital role in the development of a plant by recycling and mobilizing many important nutrients such as nitrogen, phosphorus, potassium and sulfur. These nutrients are translocated from senescing leaves to actively growing tissues, thereby supporting plants in their overall growth and reproduction (Himelblau and Amasino 2001; Hortensteiner and Feller 2002; Gregersen et al. 2013). During

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senescence, photosynthetic rates decline and the metabolism of the leaf changes which includes the degradation of chlorophylls, proteins, lipids and RNA (Matile et al. 1996; HÖrtensteiner 2006; Lim et al. 2007; Derkx et al. 2012). Leaf color changes from green to yellow during the later stages of senescence as a result of chlorophyll loss and chloroplast disassembly (Woolhouse 1984; Thomson and Platt-Aloia 1987; Zhao et al. 2012).

Senescence can be induced artificially by darkening individual leaves (Weaver and Amasino 2001), by sugars (Wingler et al. 2006) or by hormones (van der Graaff et al. 2006). This induced senescence has been studied extensively. However, gene expression patterns differ significantly between developmental and dark-or starvation induced leaf senescence (Park et al. 1998; Buchanan-Wollaston et al. 2005), and it can be assumed that at least some of the regulatory and biochemical processes involved also differ. Developmental senescence is a gradual process that is difficult to quantify. Previous studies have often used different methods to measure senescence, making a comparison of results difficult. As a consequence, the mechanisms controlling developmental senescence are still poorly understood. In *Arabidopsis*, chlorophyll content and the proportion of yellow leaves were used as indicator parameters to determine leaf and plant senescence, respectively (Levey and Wingler 2005; Balazadeh et al. 2008). The chlorophyll degradation in specific leaves, for example, leaf number 11 (Balazadeh et al. 2008), leaf numbers 5 and 6 (Hensel et al. 1993), leaf number 5 (Miller et al. 1999), the 5 largest leaves (Levey and Wingler 2005), have been proposed as measures of the senescence process. Significant differences in the onset and progress of senescence are found in different *Arabidopsis* accessions (Levey and Wingler 2005; Balazadeh et al. 2008).

It is accepted that the onset of leaf senescence is, to a large extent dependent on environmental factors (Crafts-Brandner et al. 1998; Buchanan-Wollaston et al. 2003). Progress of senescence is also influenced by external factors such as light and sugars (Weaver and Amasino 2001; Pourtau et al. 2004; Wingler et al. 2006). Nitrogen nutrition/metabolism has been recognized as an important senescence factor due to the high amount of N that is mobilized from senescing leaves to developing sink tissues (Crafts-Brandner et al. 1998). In barley (*Hordeum vulgare*) and *Arabidopsis thaliana* N deprivation resulted in accelerated leaf senescence and when additional NO_3 was supplied at the start of senescence, senescence could be halted or even reversed (Schildhauer et al. 2008).

Earlier studies on senescence to assess genetic variation in wheat (*Triticum aestivum* L.) were mainly focused on induced leaf senescence or restricted to the flag leaf (Gelang et al. 2000; Verma et al. 2004; Blake et al., 2007; Hafsi et al. 2007; Joshi et al. 2007; Gregersen et al. 2008; Falqueto et al. 2009; Srivalli and Khanna-Chopra, 2009; Chen et al. 2010; Bogard et al. 2011; Derkx et al. 2012; Naruoka et al. 2012; Zhao et al. 2012). The fast senescing flag leaf mutants of wheat had low yield, biomass and N partitioning and had less N in stem (Derkx et al. 2012).

In this study, we analysed the onset and progress of leaf and whole plant senescence in 15 doubled haploid wheat lines and their parents which were selected based on their contrasting early senescing and late senescing characters. The objectives of this research were (i) to identify parameters that can be used for the analyses of natural variation in leaf senescence of wheat; and (ii) to understand the relation between senescence development and N uptake and root traits.

Materials and methods

Plant material

The early senescing (ES) and late senescing (LS) wheat lines and their parent varieties used in the study were obtained from Directorate of Wheat Research (DWR), Karnal, India. The ES and LS lines were doubled haploids generated from HD 2329 and Camm, Indian and Australian wheat cultivars, respectively. Camm showed early senescence while HD 2329 exhibited late senescence under Indian conditions. Lines DH1 to DH8 were identified as ES, while lines DH9 to DH15 were identified as LS as based on visual observations of a large population in field studies conducted at DWR, Karnal, India.

Experimental site and growth conditions

Experiments for the present study were conducted in specialized brick structures to facilitate both above ground and below ground (root) observations (Fig. 1). Each of the brick structures with internal dimensions

Fig. 1 Brick structure used for the above ground and below ground study of ES and LS wheat lines along with their parents (**a**) and **b** shows a view of the structure being dismantled for root excavation

10 m length × 1.2 m width × 1 m height were constructed above the ground. The side walls were plastered with thin layer of cement to make it strong enough to hold sufficient soil for plant growth till maturity and also to facilitate root extraction by dismantling the bricks at any given point of time. Six such structures were used in this study. These structures were filled with top soil (15 cm depth) collected from a nearby field. The soil was 56.8 % clay, 18.0 % silt and 25.2 % sand and classified as *Typic Haplustert* with pH of 7.9, cation exchange capacity of 44.5 c mol (p+)/kg and electrical conductivity of 0.52 dS m^{-1}. The available soil nitrogen, phosphorus and potassium were 154.2, 12.77 and 530.2 kg ha^{-1}, respectively. The organic carbon concentration of the soil was 0.53 %. Before planting, the soil in the brick structures was irrigated and allowed to settle for a month.

Fertilizer required for each structure was calculated on a land area basis, at the rate of 100 kg N, 60 kg P_2O_5 and 40 kg K_2O ha^{-1} in the form of urea granules, single super phosphate and muriate of potash, respectively. Full dose of P and K and 60 % of N was applied as basal dose and the rest of N was applied 40 days after sowing.

These experiments were conducted at the farm of Indian Institute of Soil Science, Bhopal, India (23°18′48″ N latitude and 77°24′58″ E longitude). The region has a hot and sub-humid climate with mean annual rainfall of 1,083 mm and mean annual potential evapotranspiration of 1,400 mm. Much (88 %) of the rainfall occurs during the four rainy months (June to September). The mean maximum day and minimum night temperatures for the cropping period (November, December, January, February and March) were 29.0, 25.9, 25.3, 28.6, 33.6, 38.3 and 14.1, 10.9, 10.2, 12.4, 17.1, 21.8°C respectively.

Experiments were conducted twice over a period of 2 years. Initially, in the first experiment we prescreened the 15 lines along with the parents in six brick structures. Three structures were used for root studies and the other three for the measurement of yield and yield attributes. Each of the doubled haploid lines and the parents was replicated thrice. Seeds were sown on November 28, 2008 which is the ideal time for wheat sowing in this region. Irrigation was applied regularly, to ensure plants were not growing under limiting water condition. After 70 DAS, roots were excavated from 3 structures and plants in the other 3 structures were allowed to grow until maturity. Based on the results of the first experiment four lines contrasting in the onset and progress of leaf senescence were selected. In the second experiment, the four selected lines were planted in three brick structures on November 26, 2009. Each line had two rows and was replicated thrice in each of the three structures.

Chlorophyll content and leaf senescence

We followed the method described by Arnon (1949) to estimate the chlorophyll in the top 3rd and 4th fully expanded leaves of the main shoot. Powdered leaf sample (0.5 g) was immersed in 10 ml of DMSO (dimethylsulfoxide) and incubated at 65° C for 4 h. The extract was assayed in a Perkin Elmer double beam spectrophotometer for absorbance at 750, 663 and 645 nm and the chlorophyll a, b and total chlorophyll contents were determined and expressed on the basis of fresh weight. Onset of leaf senescence was defined as the day when the chlorophyll content of the leaf started to decline (similar to Levey and Wingler 2005). To determine the extent of leaf senescence we measured the chlorophyll content of the top 3rd and 4th leaves, 30 days after sowing which coincided with the first phase of leaf senescence in ES lines. Subsequently, chlorophyll measurements were made in successive leaves at 3rd and 4th position from top at every 15 days interval until 105 DAS to monitor the progress of senescence in plants. In another set of plants the third leaf from top was labeled immediately after emergence and the progression of senescence was monitored by measuring the chlorophyll content at every 5 days interval.

Yellow leaf area and whole plant senescence

The green and senescing leaf areas of ES and LS lines were measured at 15-day intervals from sowing. A leaf, which had more than 50 % of its lamina yellow, based on visual observation, was considered a senescent leaf. To measure the leaf area, shoots of two plants each of ES and LS lines were collected from each replication. The green and yellow leaves were separated, and the area was measured using a leaf area meter (LICOR-3000, LICOR Inc, Lincoln, NE, USA). The percentage of senescent leaves, which was used to define whole plant senescence, was recorded for each line over a period of 12 weeks. In order to determine the extent of plant senescence, the senescent leaf percentage at 70 DAS was considered, as it represented early boot initiation phase (Feekes scale 10) and also coincided with initiation of chlorophyll degradation in LS lines.

Leaf photosynthesis

Photosynthesis (Pn) was measured around 10.00 h in the morning using a LICOR -6400 Portable Photosynthesis System (LI-COR Inc. Lincoln, NE, USA). It was measured in contrasting ES and LS lines selected from the previous experiment. To know the Pn of leaves at different positions on the plant, measurements were made in flag leaves and penultimate leaves in ES and LS lines at booting stage. Pn of ES and LS lines was also measured in the successive leaves produced at position three and four at 15 days interval. Measurements were taken on clear sunny days when PAR ranged from 1,500 to 1,900 μmol photons m^{-2} s^{-1}. During each observation at least two measurements were made from each replication.

Phenology and grain yield

The duration from sowing to boot stage (Feekes scale is 10.1 i.e., awns visible, head is visible in the leaf sheath below the flag leaf) was recorded for all the lines. At harvest, grain yield and yield components per plant were recorded.

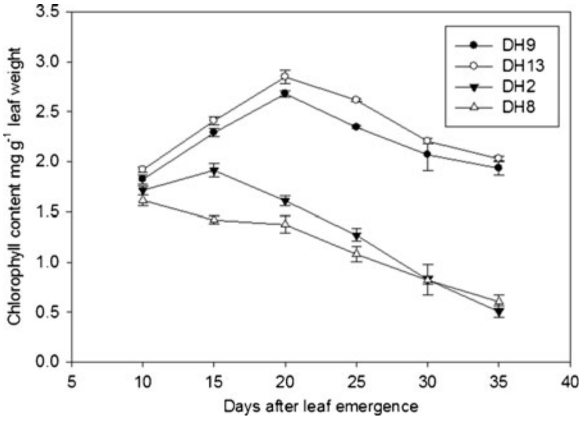


Fig. 2 Mean chlorophyll content of third leaves of early senescing (ES) and late senescing (LS) wheat lines. Each value is mean of six leaves. *Vertical bars* represent standard error. LSD ($P<0.05$) of mean values between the lines is 0.26

Root traits

The roots of wheat lines were excavated from three of the brick structures, when most of the genotypes were at booting stage (70 DAS). To retrieve the intact roots, the bricks from one side of the structure were removed (Fig. 1). The soil around the root zone was gently washed away with a jet of water until all the soil particles were removed. Care was taken to avoid the loss of secondary and tertiary roots and of root hairs during this procedure. Two plants from each replication were selected for the measurement of root and shoot properties. Roots, after separating cutting from the shoot were washed thoroughly with tap water. The moisture on the root surface was wiped with a blotting paper. The cleaned root was carefully spread on a plastic sheet to measure the maximum root length. The root volume was expressed as the amount of water displaced, when the roots were submerged in a graduated beaker. The same root was oven-dried at 70 °C for 72 h to determine the dry weight.

Then the shoot of each of the plants used for root studies was separated into green leaves, yellow leaves and stem and was dried at 80^{o}C in hot air oven to assess the above ground biomass distribution on the basis of dry weights. Wheat lines in the remaining three brick

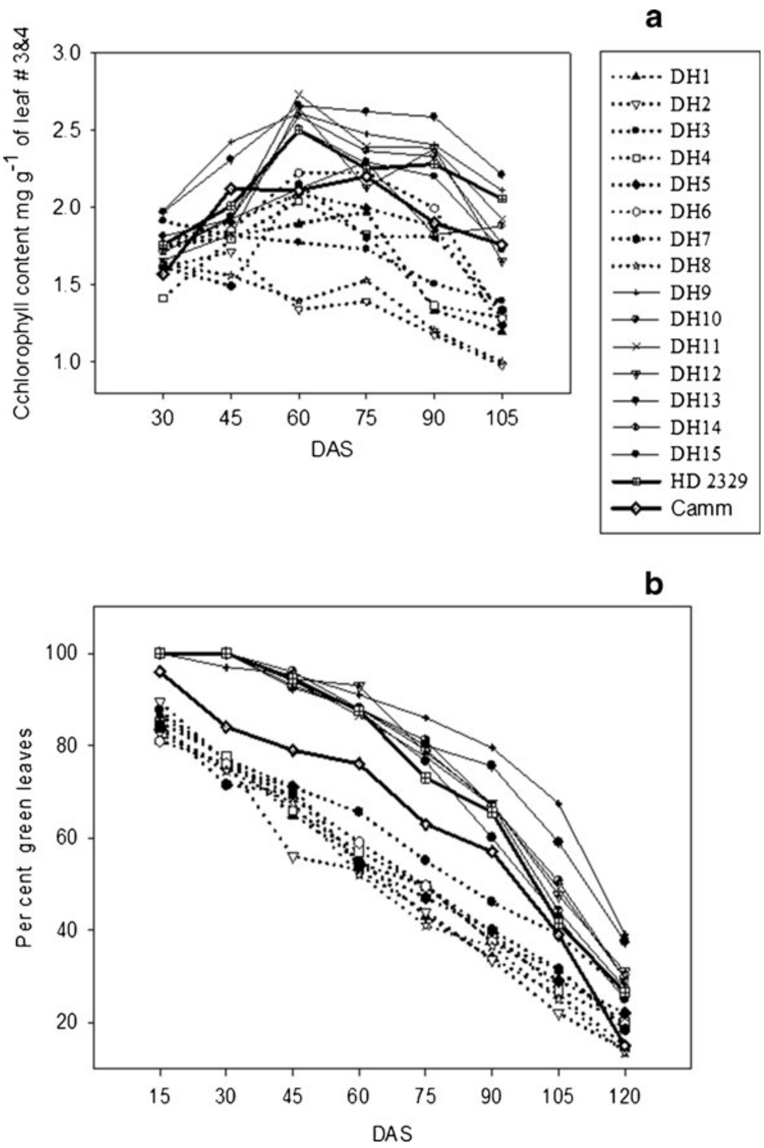


Fig. 3 Variations in leaf senescence indicators of 15 contrasting ES and LS wheat lines along with their parents: **a** chlorophyll content of leaf number 3 and 4 at different time points, and **b** percentage of green leaves measured at regular intervals after sowing. Data were mean values of six plants extracted from an ANOVA. LSD of mean values is 0.29 and 4.38 for **a** and **b** respectively

structures were allowed to grow until maturity. At maturity, grains from two plants representing a line were collected from each of the three replications. Number of grains and grain yield per plant were measured. Total biomass is the addition of root, green leaf and stem (without leaf blades) at booting and also the weight of grains at harvest.

Nitrogen content and uptake

Nitrogen (N) content of root, stem and leaves of plants collected at 70 DAS and grains collected at harvest was measured in contrasting ES (DH2 and DH8) and LS (DH9 and DH13) Lines. The oven dried (70 °C) samples were powdered with a stainless steel Wiley mill. 0.25 g of finely ground tissue sample was digested in a sulfuric acid -potassium sulfate (K_2SO_4) mixture using a selenium catalyst in a semimicro-Kjeldahl apparatus as described by Buresh et al. (1982). The N uptake of tissue was calculated by multiplying the dry weight of plant parts with their respective N concentrations. The N-uptake efficiency (UPE) - Crop N uptake per unit of N available and N-utilization efficiency (UTE) - grain dry matter yield per unit crop N uptake of each genotype was calculated as outlined by Foulkes et al. (2007).

Statistical analyses

Experimental design was a randomized complete block design with three replications Differences between wheat lines were evaluated by Analysis of variance (ANOVA) using SAS 9.2 software. Mean values were compared for significance using the F-protected LSD test at 5 % level. The relation between the senescence traits was measured by simple Pearson correlation.

Results

Natural variation of senescence in wheat lines

Wheat ES and LS lines selected for this study exhibited distinct onset and progression of senescence characters. The third leaf from top, 10 days after emergence contained 1.6 mg chlorophyll both in ES and LS lines. Chlorophyll content in LS lines increased to 2.8 mg while in ES lines it declined to 1.3 mg at 18 days (Fig. 2). Similarly, the chlorophyll content of leaves at third and fourth potions from top measured at regular interval showed a significant difference between ES and LS lines at all stages except 30 DAS (Fig. 3a). The chlorophyll content of some of the lines for example DH2 and DH8 was nearly half as that of DH9 and DH13 at 70 DAS. Between the parents significant difference was observed beyond 60 DAS. Thus, the chlorophyll content of leaves numbers three and four from top, served as leaf senescence indicator. As a measure of whole-plant senescence, the percentage of senescent and green leaves was determined for all the lines at regular intervals up to 120 DAS. The senescence commenced in ES lines as early as 15 DAS (17 % senescent leaves) and continued to increase further with age. At 70 DAS, it was almost 40 % in DH2 and DH8 (Fig. 4). During the same period the senescent leaves were negligible in LS lines.

Relation between senescence and other morphological traits

There were significant ($P<0.001$) variations between ES and LS lines for the day to booting, above and below ground biomass, yield and yield component characters (Table 1). Booting occurred 5 days earlier in ES lines than in LS lines. The grain yield of LS lines (12.8 g plant^{-1}) was more than double that of ES lines (6.3 g plant^{-1}). Genotypic variation was found to be less for grain number compared to grain weight. The test weight (100 grain weight) was as low as 1.42 g in DH8 and as high as 3.67 g in DH13. As with the grain and above ground biomass, the root biomass of ES lines was found to be 42 % less than LS lines. A similar trend was

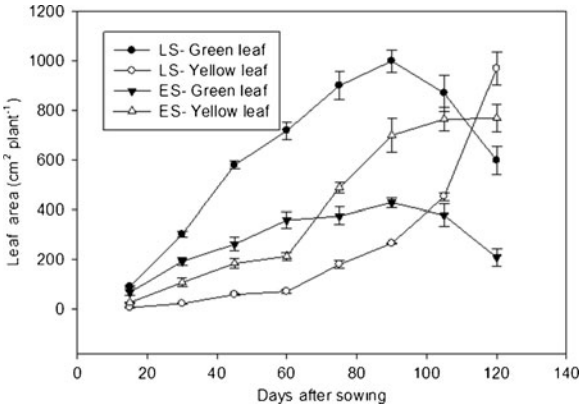


Fig. 4 Green and yellow leaf area of early senescent (ES) and late senescent (LS) wheat lines measured at an interval of 15 days. Each value is mean of nine observations. *Vertical bar* represent standard error

Table 1 Root and shoot characteristics of ES and LS wheat lines along with their parents grown in brick structure. Root, green and yellow leaf biomass, root length and root volume were collected at booting stage (70 DAS) while grain number and grain yield were collected at maturity. Each value is mean of three replications

Lines	Booting age (days)	Green leaf weight (g)	Yellow leaf weight (g)	Root length (cm)	Root biomass (g)	Root volume (cm^3)	Grain number	100 grain weight (g)	Grain yield (g)	Plant biomass (g)
Early Senescent (ES) lines										
DH 1	70	5.04	3.30	58	2.72	12.84	278	2.19	6.09	13.96
DH 2	75	4.66	4.48	65	1.71	13.03	380	1.61	6.12	13.81
DH 3	77	4.44	4.58	60	2.15	13.01	461	1.40	6.45	13.90
DH 4	76	4.41	2.72	62	2.32	14.02	363	2.09	7.59	16.02
DH 5	75	3.91	2.66	62	2.25	13.50	413	1.56	6.44	13.85
DH 6	74	4.18	3.20	60	1.84	12.92	418	1.32	5.52	12.62
DH 7	76	5.05	2.83	71	1.74	12.51	411	1.58	6.49	14.04
DH 8	73	3.67	3.75	66	1.91	12.79	417	1.42	5.92	12.38
Camm	76	3.82	3.70	62	2.77	12.93	413	1.40	6.05	12.64
Mean	74	4.40	3.47	63	3.04	13.06	394	1.65	6.30	13.69
Late senescent (LS) lines										
DH 9	76	12.09	0.88	50	4.93	39.54	528	3.09	16.32	37.94
DH 10	85	9.44	1.32	58	3.33	46.96	499	2.86	14.27	30.67
DH 11	88	8.98	1.75	55	2.88	45.97	459	2.36	10.83	25.78
DH 12	67	10.46	1.12	55	3.01	45.95	393	2.86	11.24	27.65
DH 13	74	11.66	0.95	53	5.08	48.38	494	3.67	18.13	38.17
DH 14	89	10.34	1.55	59	2.85	46.14	577	2.65	15.29	31.77
DH 15	89	10.97	1.19	60	3.60	45.73	395	2.66	10.51	27.20
HD 2329	77	10.97	1.19	62	5.71	46.03	398	2.33	12.77	27.94
Mean	79	9.96	1.04	57	6.38	45.58	459	2.68	12.80	28.98
LSD ($P<0.05$)										
Lines	5	1.22	0.55	5	0.74	2.27	NS	0.22	1.80	2.14
ES vs. LS	**	***	***	***	***	***	NS	***	***	***

observed for root volume which was 71 % less than LS lines. On the other hand, the length of the longest root was more in ES lines (63 cm) than in LS lines (57 cm).

The Pearson correlations revealed significant associations between the extent of senescence and duration for booting, root length, root biomass, root volume, grain yield or chlorophyll content (Table 2). The correlation was significantly negative for booting duration, root biomass, root volume, grain yield, chlorophyll content while, it was significantly positive for length of the longest root at booting stage.

Variations in morphological and physiological traits

Experiment II confirmed the difference in leaf senescence pattern between ES and LS lines with almost 33 % senescent leaf area in 15 day old young seedlings of ES lines while it was negligible in LS lines up to 60 DAS (Fig. 4). At post anthesis phase, there was significant increase in yellow leaf area of LS lines.

The chlorophyll, Pn and N concentration measured in different leaves from top revealed large differences between ES and LS lines (Fig. 5). Flag leaves and penultimate leaves did not show significant differences; however all other leaves down the canopy profile had chlorophyll, Pn and N significantly lower in ES lines than LS lines. The chlorophyll, Pn and N content of third leaves of ES lines were 48 %, 54 % and 30 % lower compared to flag and 2nd leaves. The mean Pn and N concentration measured in leaf numbers 3 and 4 positioned from top (Fig. 6) were significantly low in ES lines at all growth stages. Root biomass was significantly low in ES lines and this was also reflected in the low root to shoot biomass ratio in ES lines (9.5 %) as against 20 % in LS lines (Fig. 7).

The N concentration and N content of root, stem, leaf and grains were distinctly low in ES lines (Table 3). However, the variation in concentration was less significant compared with the content of N between ES and LS lines reflecting a large difference in overall plant biomass. The plant N uptake of ES lines was poor (255 g) compared to LS lines (676 g). As a consequence, the N uptake efficiency of ES lines was only 0.19 as against the 0.27 of LS lines. On the other hand the N utilization efficiency was higher in ES lines (25) compared to LS lines (21). The Fig. 8 also indicated that the extent of plant senescence was negatively correlated with plant N uptake (Fig. 8a) and also with root biomass (Fig. 8b), while the plant N uptake had a strong positive correlation with root biomass (Fig. 8c).

Discussion

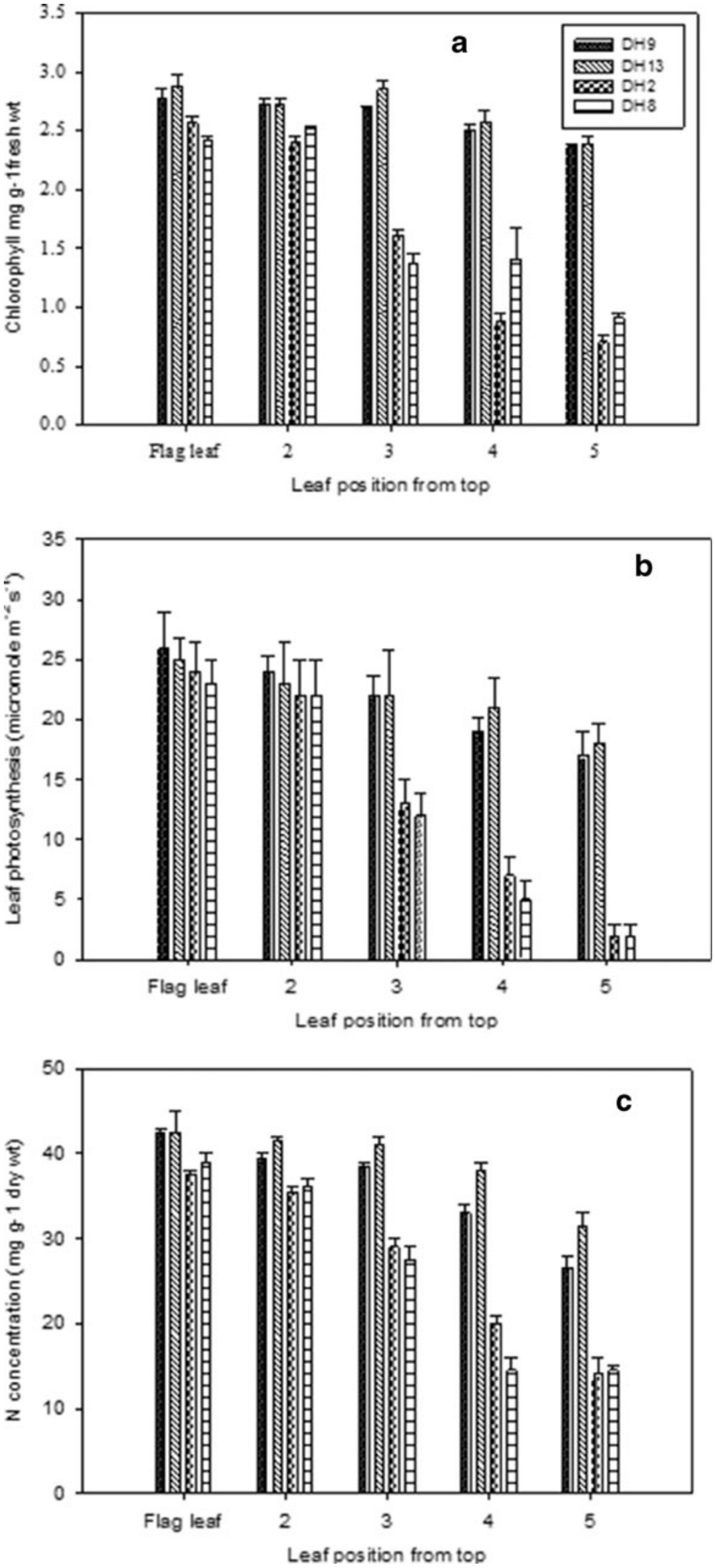
The contrasting early senescent and late senescent doubled haploid progenies of Australian and Indian wheat varieties, used in this study were selected from a field experiment conducted at Directorate of Wheat Research (DWR), Karnal, India. These lines when grown in specialized brick structures designed for root studies exhibited distinct phenotypic traits, accelerated and normal development of senescence. Field phenotyping for root traits is otherwise difficult. The brick structures used in this study not only enabled us to identify the genotypic

Table 2 Correlation between senescence and other morphological traits of early senescing (ES) and late senescing (LS) wheat lines. The table shows the Pearson correlation coefficient *,**, and *** indicate significant correlations at $P<0.05$, 0.01 and 0.001 respectively

Trait	Booting age	Root lenght	Root weight	Root volume	Grain number	100 grain weight	Grain yield	Chlorophyll
Extent of senescence*	−0.469**	0.707**	−0.794***	−0.925***	−0.454**	−0.852***	−0.922***	−0.782***
Booting age		0.061	0.288	0.109	0.375	0.225	0.310	0.214
Root lenght			−0.8***	−0.78***	−0.359	−0.738**	−0.724**	−0.608***
Root weight				0.926***	0.45	0.916***	0.927***	0.732***
Root volume					0.628**	0.912***	0.963***	0.610***
Grain number						0.41	0.626***	0.450**
100 grain weight							0.943***	0.688***
Grain yield								0.675***

*The extent of senescence is the percentage of senescent leaf area measured at 70 DAS; while the chlorophyll content represents the mean content of third and fourth leaf

Fig. 5 Influence of leaf position on **a** Chlorophyll, **b** photosynthesis, and **c** N content of early senescent (ES) and late senescent (LS) wheat lines measured at the booting stage. Each value is mean of six values. *Vertical bar* represent standard error. LSD values between the lines is 0.8, 7.0 and 6.5 for **a**, **b** and **c** respectively



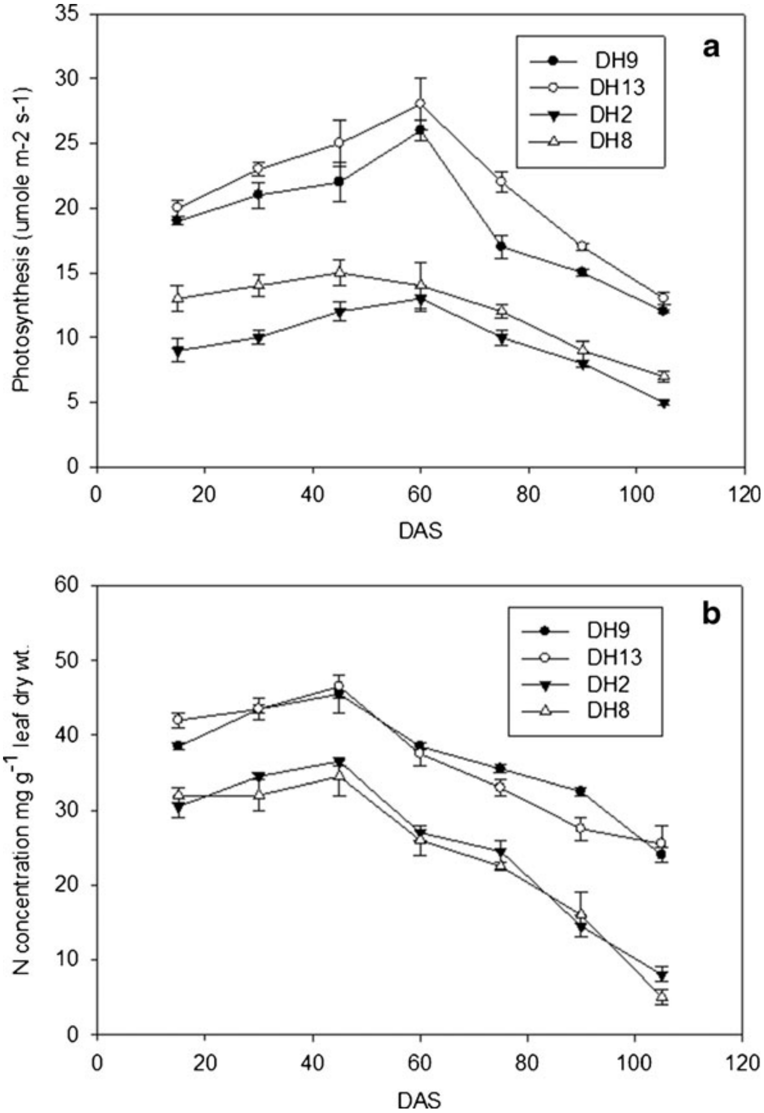


Fig. 6 Mean photosynthesis and N concentration of leaf number 3 and 4 from *top* of early senescent (ES) and late senescent (LS) plants measured at different time points. Each value is mean of six values. *Vertical bar* represent standard error

variation in senescence traits but also made it possible to study the association between the shoot and root in the development of senescence.

In field grown wheat, the senescence of older leaves is common at post anthesis phase (Munné-Bosch and Alegre 2004) as seen in LS lines grown in brick structure. Based on the chlorophyll content of leaf number 3 and 4 and the extent of plant senescence, we could clearly differentiate early senescing and late senescing lines. Natural genetic variation is recognized as an important resource for plant biology analysis and has been used extensively in model species such as *Arabidopsis* (Alonso-Blanco and Koorneef 2000). Despite this interest, data on natural variation in wheat senescence are still limited. The two main obstacles are gradual progress of senescence and a lack of well defined markers to monitor leaf and/or plant senescence in lines having different phenology and duration. We also measured several morphological and physiological parameters to study the correlations between the senescence indicators. Our results demonstrate a significant correlation between the onset and extent of whole plant senescence and booting age, grain yield, root length, root biomass, root volume and chlorophyll content. In general lines with early senescence behavior enter the reproductive phase of the development faster, produced leaves with low chlorophyll content and less grain yield. Similar results were reported for Arabidopsis accessions studied by Levey and Wingler (2005). In addition the ES lines

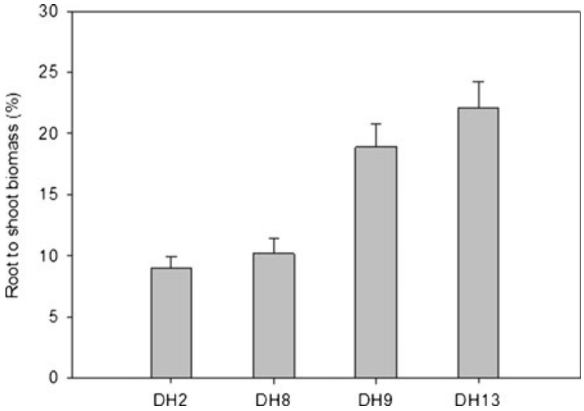


Fig. 7 Root to shoot biomass of early senescent (ES) and late senescent (LS) wheat lines measured at booting stage (70DAS). Each value is mean of six values. *Vertical bar* represent standard error

produced less root biomass (as indicated in weight and volume) compared to LS lines.

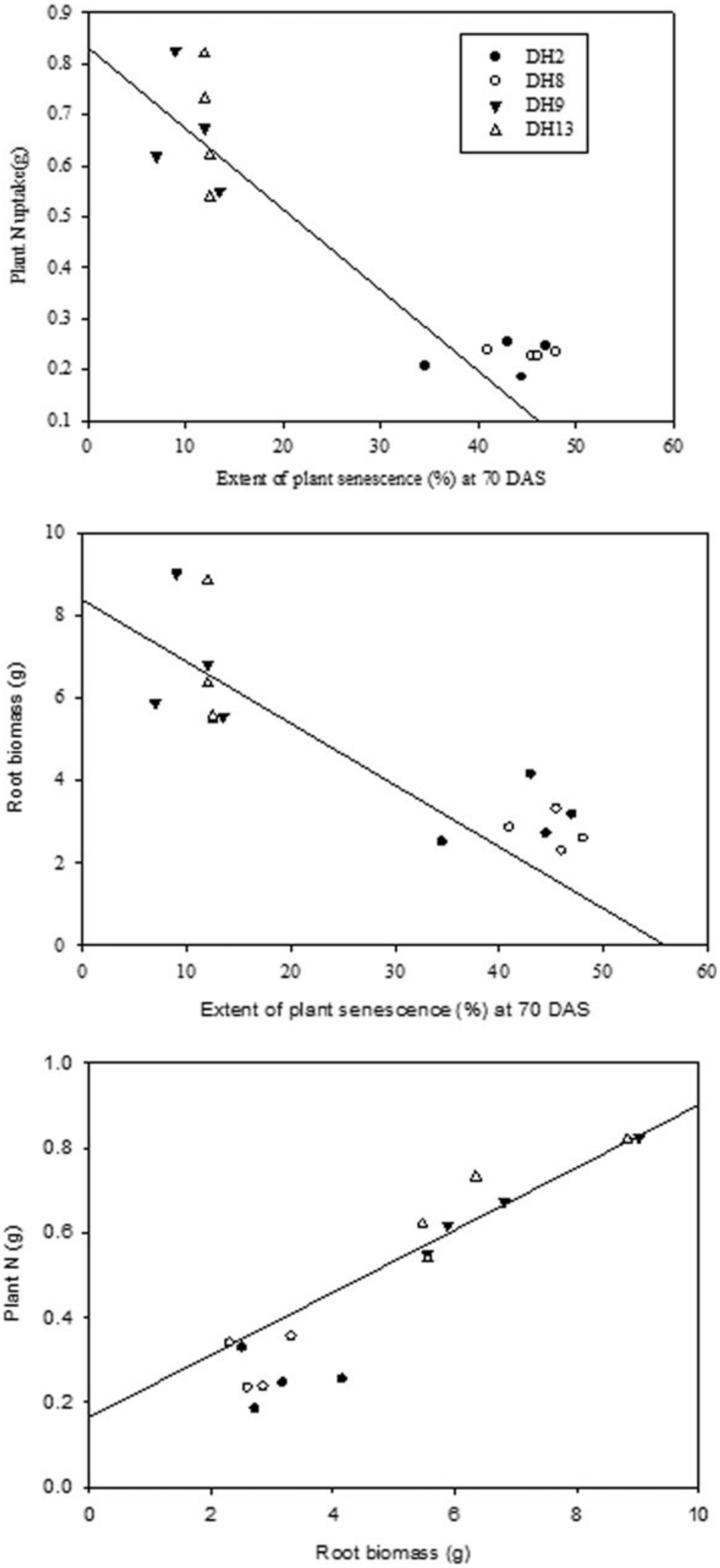
The results obtained in this study supported our hypothesis, that there was an association between root system and the onset and progression of leaf senescence. Using the specialized brick structure we designed, we could precisely measure the root properties across the wheat lines. It was measured in 70 day old plants (boot stage), because in wheat, at this stage the length and biomass of root was found to be maximal (Gregory et al. 1978; Barraclough and Weir, 1988). There was a distinct difference between the root systems of ES and LS lines. In fact, we observed deeper roots for ES lines in brick structure experiments. However, they had only few lateral roots and root hairs which were reflected in their root volume and biomass. On the other hand, the LS lines had more prolific root system characterized by high root volume, more lateral roots and root hairs. Root and root hair growth are found to be influenced by both genetic and environmental factors (Wiesler and Horsi 1994). However, in our study the difference observed in root architecture of ES and LS lines is purely genetic, as these lines were grown under identical weather and soil conditions. The senescence development in ES lines was precocious and was constitutively expressed trait. This understanding and the lines identified may be worth to study the relevance and rate of senescence development under different abiotic stress situations.

For a comparative analysis of ES and LS lines to the physiological and nutrient uptake studies, we focused on contrasting early senescing (DH2 and DH8) and late senescing (DH9 and DH13) lines with distinct variation for root traits like rooting depth, root mass and rooting volume, which are important for acquisition of nutrients from soil (Foulkes et al. 2007; Van Noordwijk, 1983). N uptake is known to be improved in genotypes with abundant root branching (Fillery and McInnes 1992, Liao et al. 2004). In our study, we observed that the total plant N of ES lines was less than half that of LS lines, even when the supply was the same. This was mainly due to poor N uptake efficiency rather than utilization efficiency. N utilization efficiency, which depends on N efficiency of biomass formation, the effect of N on carbohydrate partitioning, nitrate

Table 3 N concentration and N content in different plant parts, N uptake efficiency (UPE=plant N/available N) and N utilisation efficiency (UTE=grain yield/plant N). (Mean values followed by same letter within columns are not significantly different ($P<0.05$) according to LSD test

Lines	Nitrogen concentration (mg g^{-1})					Nitrogen content (g)						UPE	UTE
	Root	Stem	Green leaf	Yellow leaf	Grain	Root	Stem	Green leaf	Yellow leaf	Grain	Plant		
DH 2	15.5b	9.5b	13.5b	7.7b	15.5b	49b	16b	62b	28	95b	250	0.19b	26.27a
DH 8	17.2ab	12.0ab	13.5b	10.2b	17.5b	48b	18b	53b	31	106b	256	0.18b	26.63a
DH 9	19.7a	13.2a	17.7a	19.7a	22.2a	137a	37a	186a	26	294a	680	0.35a	19.33b
DH 13	19.2a	13.0a	18.5a	20.5a	20.5a	126a	37a	203a	22	268a	656	0.35a	19.90b
ES	16.4	10.8	13.5	9	16.5	49	17	58	30	101	255	0.19	24.95
LS	19.5	13.1	18.1	20.1	21.0	130	37	194	24	281	676	0.27	21.48
LSD													
Lines	3	2.6	2.8	2.8	2.8	4.0	9.0	23	NS	76	81	0.07	3.11
ES vs. LS	**	**	**	***	***	***	***	***	NS	***	***	***	***

Fig. 8 Correlations between the extent of plant senescence and **a** plant N uptake, $R^2=0.872^{***}$; and **b** root biomass, $R^2=0.746^{***}$; and between **a** root biomass and plant N uptake, $R^2=0.931^{***}$. ***indicates significant at $P<0.001$ level



reduction efficiency and remobilization of nitrogen from senescence tissue as well as storage function (Lea and Azevedo 2007, Hirel et al. 2007), was significantly higher in ES lines compared to LS lines. This study clearly suggests that the ES lines are efficient in utilizing the available nutrients; however they are inefficient in the uptake of nutrients mainly because of poor root growth. The strong negative correlation observed between the extent of plant senescence and plant N uptake and also with root biomass further confirmed that the poor root growth restricted uptake of N led to the onset and progression of senescence in ES lines.

Conclusions

There were considerable variations in senescence in different doubled haploid progenies of wheat, with respect to the onset and progression of senescence. The senescence commenced in ES lines within few days of full expansion of the third leaf from the top, whose chlorophyll, photosynthesis and N concentration was significantly low compared to LS lines. These ES lines also had poor root growth. The strong negative correlation observed between the extent of plant senescence and plant N uptake and also with root biomass suggested that the poor root growth restricted uptake of N led to the onset and progression of senescence in ES lines. The senescence development in ES lines was precocious and was constitutively expressed trait. This understanding and the lines identified may be worth to study the relevance and rate of senescence development under different abiotic stress situations, as well as their ability to mobilize the nutrients under adverse situations. The lines selected by us had constitutive expression of leaf senescence trait under no stress conditions and they could be very useful in understanding the genetic regulation of senescence and could be useful genetic markers if any trait is associated.

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