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Full Length Article

Effect of Selenium on Carbon Partitioning and Nitrogen Allocation at Three Developmental Stages in Oilseed Rape (*Brassica napus*) Plants

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Abstract

In order to investigate the mechanisms for the effect of selenium (Se) on leaf senescence, oilseed rape plants were grown under growth chamber conditions in perlite irrigated with nutrient solution and harvested at three distinct developmental stages; rosette, bolting and fruiting stages. Se treatment was started for six-week-old plants with 3 µg (as Na_2SeO_4) L^{-1} $week^{-1}$, reaching the total amount of 50 µg Se L^{-1} in the plants harvested at fruiting stage. At harvest, the young, middle-aged and old leaves were analysed separately for relative chlorophyll concentration, photosynthesis and respiration rate, carbohydrates and proteins, nitrogen, phosphorus and potassium concentrations. Results showed that leaf photosynthesis and respiration rate were both consistently elevated by Se, irrespective of the leaf age and of the developmental stage of the plant. Senescence of old leaves was postponed by Se at rosette and bolting, but not at fruiting stage. Se exerted a dual effect on nitrogen allocation to and remobilisation from the old leaves. At rosette and bolting stages, Se increased nitrogen allocation to the old leaves and diminished its remobilisation, whereas accelerated nitrogen remobilisation towards reproductive parts at fruiting stage. Relative leaf chlorophyll concentration was positively correlated with the extent of nitrogen but not of phosphorus or potassium remobilisation, suggesting involvement of nitrogen remobilisation in Se-mediated delay of leaf senescence.

Keywords: Carbohydrates; Protein; Rosette; Bolting; Fruiting; N remobilisation

Introduction

In annual plants, completion of the entire life cycle is correlated with leaf senescence (Lim *et al.*, 2007; Thomas, 2013). Senescence is an integral part of plant development and is subject to regulation by many environmental and autonomous factors (Gregersen *et al.*, 2013). In monocarpic species belonging to the Gramineae, the onset of leaf senescence is closely correlated with seed development. In members of the Brassicaceae, by contrast, leaves mature and senesce independently of the reproductive cycle (Liu *et al.*, 2008). In these species, each leaf reaches maturity following a period of peak photosynthetic activityand after a period as a source organ, they begin to senesce. Upon flowering, the formation of leaves stops and nutrients areas simulated and relocated to the reproductive structures. Ultimately the loss of photosynthetic capacity due to leaf senescence leads to organ death, which is closely correlated with the production of mature seeds (Zentgraf *et al.*, 2004).

Selenium (Se) is a beneficial element for higher plants. Its role in the enhancement of plant tolerance to various environmental stress factors such as drought, salinity and UV radiation has been well documented (Hajiboland, 2012; Feng *et al.*, 2013). Under optimum conditions, growth is also promoted by Se, particularly in Brassicaceae, that is mainly associated with activation of carbon (C) and nitrogen (N) metabolism (Malik *et al.*, 2011; Hajiboland and Sadeghzadeh, 2014). It has been observed that Se at low concentrations increases net photosynthesis, nitrate assimilation and protein synthesis (Hajiboland and Sadeghzadeh, 2014). Although Se considerably stimulates leaf photosynthesis rate up to 2-fold, accompanied by higher carbohydrates content, dry matter production is much less affected (Hajiboland *et al.*, 2014; Hajiboland *et al.*, 2015). It is plausible that Se influences the plant's C budget and alters its partitioning to biomass or metabolic pools rather than increasing the photosynthesis rate alone.

Selenium has been known to delay senescence in some plant species such as lettuce (Xue *et al.*, 2001) and soybean (Djanaguiraman *et al.*, 2005). An antioxidative effect of Se has been suggested as the main mechanism for its anti-ageing effect (Djanaguiraman *et al.*, 2005). In oilseed rape plants, we observed previously that Se accelerates flowering while it delays fruit set so that siliques remain green longer in the Se-treated plants (Hajiboland and Keivanfar, 2012). In an experiment with detached leaf, we demonstrated recently that Se delays senescence via maintaining the photochemical capacity of the leaf, not only during natural ageing but also under N deficiency-induced senescence (Rahmat *et al.*, 2017).

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The progression of leaf senescence is usually evaluated by different physiological indicators such as leaf yellowing or change in protein and/or chlorophyll contents (Levey and Wingler, 2005). As with other types of programmed cell death, plant senescence is accompanied by a decrease in protein synthesis and upregulation of genes involved in senescence (Gombert *et al.,* 2006). During leaf senescence, considerable changes occur in the nutrient content of leaves. Mature and old leaves are characterised by loss of main nutrients particularly N (Hörtensteiner and Feller, 2002; Guiboileau *et al.,* 2010). Indeed, one of the most conspicuous events during sequential leaf senescence is protein degradation and N remobilisation into active sinks such as young leaves and/or reproductive organs (Avice and Etienne, 2014).

Oilseed rape (*Brassica napus* L.) is the second and third main crops for feed and food, respectively. Timing of senescence in relation to C capture and nutrient remobilisation is a major determinant of the yield in this crop species (Avice and Etienne, 2014). In this work, in order to investigate the mechanisms for Se-mediated delay of leaf senescence, C partitioning and N allocation to the leaves of different age was studied in this crop species during three subsequent developmental stages.

Materials and Methods

Oil seed rape (*Brassica napus* L. cv. RGS) plants were grown from seeds and cultivated in perlite irrigated with nutrient solution without (–Se) or with Se (+Se) treatment under growth chamber conditions for about 3 months until fruiting stage. Plants were harvested three times at one-week intervals within three distinct developmental stages. At each harvest time, after some non-destructive measurements such as leaf relative chlorophyll, photosynthesis and respiration rates, one group of similar plants was destructively harvested and analyzed for various physiological parameters.

Plant Culture and Treatments

Seeds provided by the Seed and Plant Improvement Institute (Karaj, Iran), were surface sterilised and germinated in the dark on perlite. After germination, seedlings were transferred to the light. Ten-day-old seedlings were transferred into 1.5 L pots (two plants in each pot) filled with perlite and irrigated with Hoagland nutrient solution (pH 5.8–6.0) or water at intervals. The volume of nutrient solution used for irrigation of pots started with 100 mL pot^{-1} $week^{-1}$ and gradually increased to 250 mL pot^{-1} $week^{-1}$ during further growth period. Selenium treatment (as Na_2SeO_4) was started four weeks after transplanting. Plants were treated with 5 µg Se pot^{-1} $week^{-1}$ (~3 µg Se L^{-1} $week^{-1}$), reaching the total amount of 75 µg Se pot^{-1} (50 µg Se L^{-1}) in the plants harvested at fruiting stage. The level of Se treatment was selected according to our previous work (Hajiboland and Keivanfar, 2012). Plants were grown under controlled environmental conditions with a temperature regime of 25°/18°C day/ night, a 14/10 h light/dark period and relative humidity of 50–60%, and at a photon flux density of about 400 µmol m^{-2} s^{-1}.

Harvest

Plants were harvested three times at one-week intervals within three distinct developmental stages using BBCH (Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie) scale, including rosette (BBCH 16–17, 20–22 and 23–26), bolting and flowering (BBCH 32–35, 52–55 and 62–65) and fruiting and senescence (BBCH 73–76, 79–82 and 84–88) stages. Three distinct shoot fractions were defined and analysed separately at each developmental stage, including young leaves, middle-aged leaves and old leaves (Fig. 1). Each fraction consisted of three leaves, except young leaves that may include more than three leaves following emergence of leaves during three weeks.

At each developmental stage, gas exchange parameters (at all three harvests) and leaf relative chlorophyll concentration (at 3rd harvest) were determined in the leaves of different age, as defined above. Leaf relative chlorophyll concentration was reported as SPAD (Soil Plant Analysis Development) readings using a portable chlorophyll meter (Minolta, SPAD 502). Net CO_2 assimilation rate was measured with a calibrated portable gas exchange system (LCA-4, ADC Bioscientific Ltd., UK) between 10:00 and 13:00 a.m. at a photosynthetic photon flux density of 350 µmol m^{-2} s^{-1}. Dark respiration was determined on the subsequent day between 02:00 and 04:00 a.m. with the same gas exchange system in the same leaves. All measurements were made under relative humidity of 70–80%, leaf temperature of 23–25°C and CO_2 concentration of 370–410 mg kg^{-1} provided in the leaf chamber of gas-exchange unit. The net photosynthesis or respiration rate per unit of leaf area (µmol CO_2 m^{-2} s^{-1}), was calculated automatically by the gas exchange system using the values of CO_2 and humidity variation inside the chamber, both measured with the infrared gas analyser.

After measurement of leaf SPAD and gas exchange parameters, plants were harvested. Shoot and roots were separated, washed with distilled water and blotted dry on filter paper. After determination of shoot and roots fresh weight, samples were oven-dried at 70°C for 48 h and dry weight (DW) was determined and used for carbohydrates, proteins and elemental analyses.

Measurement of Soluble Sugars and Proteins

For determination of soluble sugars, 20 mg of dried ground plant materials was extracted twice with 2.5 mL 80% ethanol in a water bath for 2 h at 30ºC. After centrifugation at 3,000 g for 10 min, the resulting supernatants from both extraction steps were combined and subjected to soluble sugars analysis by anthrone-sulfuric

reagent at 630 nm and standard curve was created using glucose (Quentin *et al.*,2015).

For determination of total soluble proteins, 200 mg of oven-dried samples were mixed with 10 mL of 0.1 N NaOH and incubated at 70ºC for 30 min in a water bath. Soluble proteins were extracted from the mixture twice after centrifugation for 10 min in 3,000 g. The supernatants were combined and subjected to analysis by Bradford method (1976) using a commercial reagent (Sigma) and BSA (Merck) as standard.

Determination of Root Respiration Rate

Reduction of triphenyltetrazolium chloride (TTC) (Sigma) by root tissues to the red-colored insoluble triphenylformazan that is analogous to the activity of the mitochondrial respiratory chain was measured in the fresh roots according to the method described by Ruf and Brunner (2003). This analysis was performed only for plants at rosette stage harvested at 3rd week.

Determination of Nitrogen, Phosphorus and Potassium

Dry samples of 50 mg were wet-digested using perchloric acid for 2–3 h at 250ºC – 300ºC. The ash was dissolved in HCl and made up to volume with distilled water. Concentration of N was determined using the indophenol blue method, according to the method described by Koroleff (1983). The reaction solution including an alkaline medium (containing 68 mM tri-sodium citrate and 12.5 mM NaOH) and 5% sodium hypochlorite (fresh commercial bleach) as oxidising reagent was mixed with 100 µL of sample. For development of a blue colour, 160 µL of phenol-alcohol solution (100%, w/v) was added to the mixture and incubated for 1 h in the dark. The absorbance was determined at 630 nm and standard curve was created using NH_4Cl (Merck) over the range of 0–100 ppm (Koroleff, 1983). In addition to N, concentration of P was determined using ammonium molybdate-vanadate colorimetric method, and concentration of K was determined by flame photometry.

Experimental Design and Statistical Analyses

This experiment was undertaken in a complete randomised block design with four replications. A three-way analysis of variance (ANOVA) was performed in which levels of developmental stages (rosette, bolting and fruiting), harvest intervals (1st, 2nd and 3rd week) and Se treatment (–Se and +Se) were included. Data of parameters recorded only at one harvest time, were analyzed by a two-way ANOVA. Comparison of two Se treatments was performed using *t*-test ($p \leq 0.05$). The Sigma stat (3.02) package was used for ANOVA, *t*-test and regression analyses performed between SPAD and % changes in the N, P and K content of the leaves.

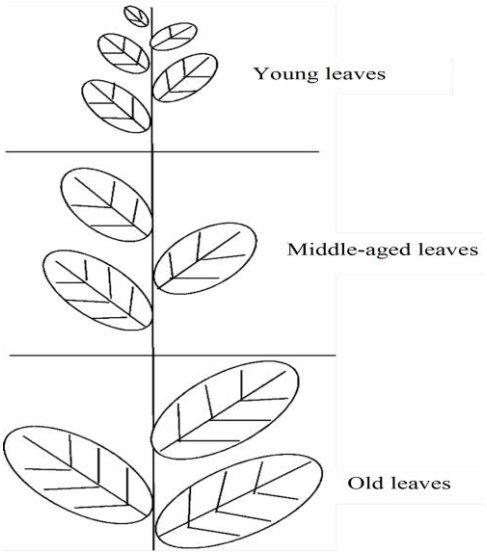

Fig. 1: Schematic illustration for designation of leaves of different age harvested and analyzed separately during three subsequent harvests at each developmental stage. It is noteworthy that, three defined age groups remained the same within each developmental stage but were different between different stages

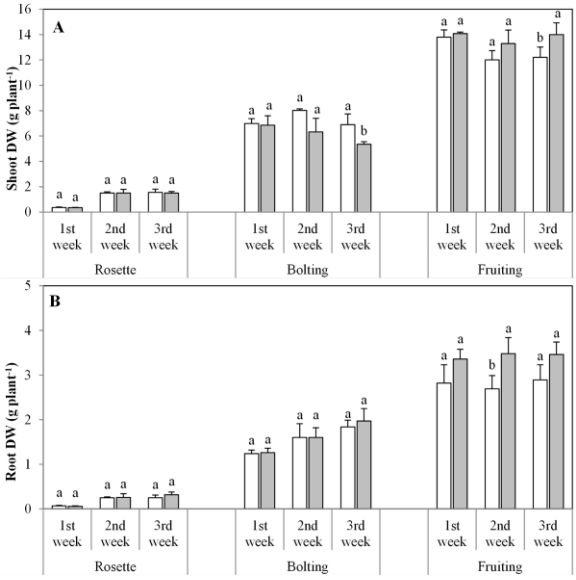

Fig. 2: Shoot (A) and root (B) dry weight in the absence (−Se) or presence of Se (+Se) during three subsequent weeks within three developmental stages in oil seed rape plants. Bars indicated by the same letter are not significantly different (*t*-test, $p \leq 0.05$)

Results

Shoot and root DW were not influenced by Se treatment at rosette stage. At bolting stage, however, Se treatment rather decreased shoot (but not root) DW by about 22% that was

significant at 3rd harvest ($p \leq 0.05$). A slight or significant ($p \leq 0.05$) increase of shoot and particularly root DW was observed in plants at fruiting stage (Fig. 2).

In the absence of Se, net photosynthesis rate was decreased during three subsequent harvests in the leaves of plants at rosette and bolting stages. In plants at fruiting stage, however, after a transient decline, photosynthesis rate was increased again in the leaves of different age. Although the same trend of changes was observed in Se-treated plants during three subsequent weeks and at three developmental stages, +Se plants showed a considerably higher rate of photosynthesis, observed particularly in the lower leaves with an increase of 57–96% (Fig. 3).

Respiration rate of –Se plants at rosette and bolting stages transiently increased in the time between the first two subsequent weeks while either decreased or remained stable at 3rdweek irrespective of the leaf age. In plants at fruiting stage, however, weekly measurement of respiration revealed a steady increase in the young leaves, an increase after a transient reduction in the middle-aged leaves and stable levels in the old leaves. The same trend was observed in +Se plants with slightly or significantly higher respiration than −Se plants in the leaves of different age at all developmental stages (Fig. 4). Root respiration rate was also slightly higher in +Se plants (Fig. 5).

Leaf SPAD values increased significantly ($p \leq 0.05$) with Se treatment in the old leaves at rosette and bolting (but not at fruiting) stages. Effect of Se on middle-aged leaves was negligible and its effect on the young leaves was significant only at bolting stage (Table 1).

Concentration of soluble sugars increased upon Se treatment at rosette stage particularly in the old leaves showing an increase of about 123%. In plants at bolting stage, however, although soluble sugars concentration was consistently higher, a significant effect of Se was observed only in the old leaves. At fruiting stage, soluble sugars concentration either did not respond to or was decreased by Se. Root concentration of soluble carbohydrates increased slightly or significantly with Se except in plants of the last harvest at fruiting stage (Table 2).

Protein concentration of leaves was slightly or significantly ($p \leq 0.05$) higher in +Se plants at rosette stage. At bolting stage, however, effect of Se was different in the leaves of different age. In the young leaves, slight decrease in protein concentration was observed upon Se treatment while slight or significant increase of protein concentration was found in the middle-aged and old leaves. At fruiting stage, a consistent (slight or significant) reduction of protein concentration was observed in Se-treated plants in the leaves of different age. Root protein concentration was higher in +Se plants at rosette and fruiting stages, while it decreased upon Se treatment in plants at bolting stage (Table 3).

Relative (%) distribution of N into different shoot fractions was influenced by Se at rosette and bolting stages.

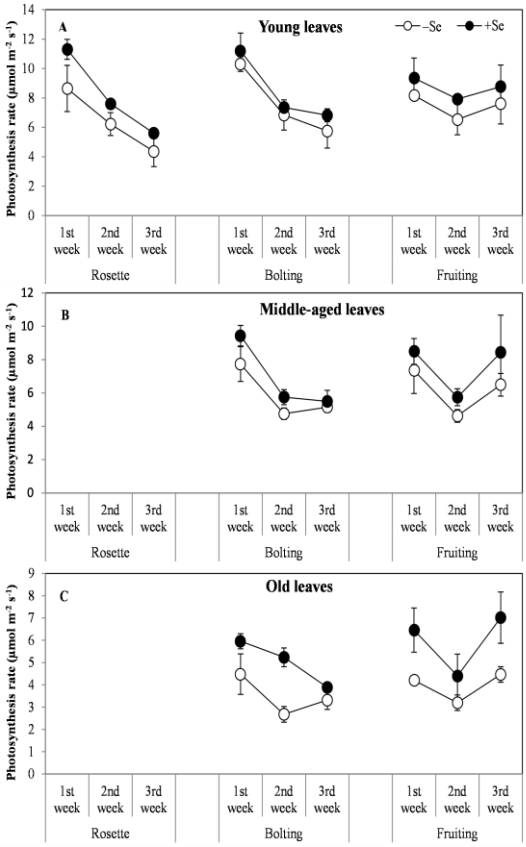

Fig. 3: Net assimilation rate in the absence (−Se) or presence of Se (+Se) in the leaves of different age during three subsequent weeks within three developmental stages in oil seed rape plants

Allocation of N to the old leaves increased significantly ($p \leq 0.05$) while it was lowered by Se in the young leaves and remained unchanged in the middle-aged leaves. Effect of Se on N allocation into roots was not consistent and mainly non-significant (Table 4).

Young leaves lost N at rosette stage while at bolting and fruiting stages, N content of these leaves was increased during three subsequent weeks. Old leaves, in contrast, lost N during both rosette and bolting stages as an indication of remobilisation into younger leaves during three weeks growth (Fig. 6). At fruiting stage, N content of both young and old leaves rather increased during three subsequent weeks. Effect of Se treatment depended on leaf age and developmental stage. At bolting stage, it inhibited N remobilisation that was reflected in an increase of N content in both young and old leaves. At rosette stage, it either accelerated (in the young leaves) or inhibited (in the old leaves) the N remobilisation. At fruiting stage, the % N change was not affected by Se in the young leaves, while decreased by Se in the old leaves (Fig. 6). Changes in the P content of the old leaves were lower in +Se plants at all three developmental stages while for K, it was rather increased by Se treatment (Fig. 7).

Table 1: Leaf SPAD data as influenced by developmental stage of plants in the absence (−Se) or presence of Se (+Se) in oilseed rape plants. Different letters between two Se treatments indicate statistically significant difference (*t*-test, $p \leq 0.05$)

Treatment	Rosette		
	Young L	Middle-aged L	Old L
–Se	32.2±1.8 [a]	27.8±1.9 [a]	15.2±1.4 [b]
+Se	31.5±2.2 [a]	29.4±1.3 [a]	25.1±1.6 [a]
		Bolting	
–Se	53.3±2.4 [b]	44.3±3.7 [a]	25.9±3.6 [b]
+Se	61.2±0.7[a]	46.4±1.4 [a]	40.1±4.3 [a]
		Fruiting	
–Se	44.4±1.2 [a]	39.6±1.2 [a]	29.3±3.7 [a]
+Se	46.8±2.4 [a]	40.3±0.3 [a]	29.9±1.6 [a]

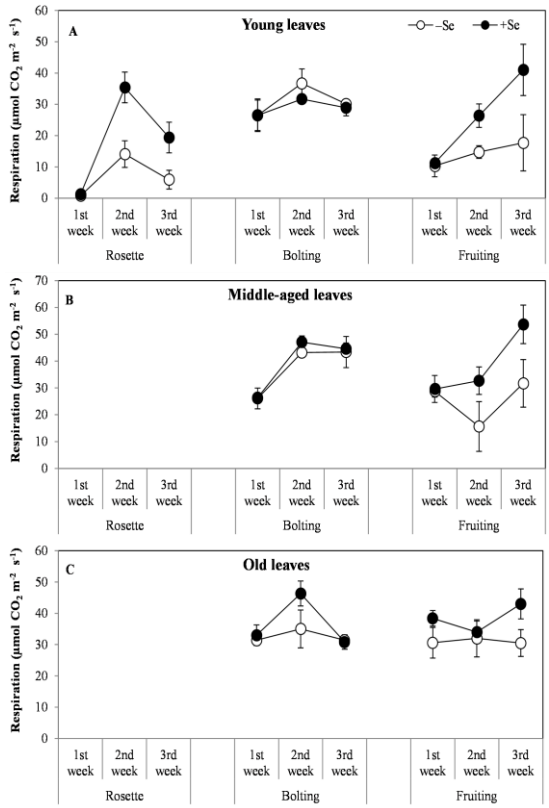

Fig. 4: Dark respiration in the absence (−Se) or presence of Se (+Se) in the leaves of different age during three subsequent weeks within three developmental stages in oil seed rape plants

A significant ($p \leq 0.001$) correlation was observed between % N change and leaf SPAD values irrespective of the leaf age and developmental stage, particularly in +Se plants (Fig. 8). However, a negative relationship ($p \leq 0.05$) was found between SPAD values and % changes in the P and K content (Fig. 8).

Results of ANOVA (Table 5) indicated that developmental stage of plants influenced the majority of parameters except for photosynthesis of the middle-aged leaf and respiration of the old leaves. Harvest time did not influence shoot DW and N allocation to the middle-aged leaf. Similarly, Se treatment significantly influenced the majority of parameters except shoot DW and N allocation to the middle-aged leaf.

Table 2: Concentration of soluble carbohydrates (mg g^{-1} DW) in different shoot fractions and roots as influenced by the developmental stage of plants in the absence (−Se) or presence of Se (+Se) during three subsequent weeks in oilseed rape plants. Different letters between two Se treatments indicate statistically significant difference (*t*-test, $p \leq 0.05$). n.d., not determined

Harvest	Treatment	Rosette			
		Young L	Middle-aged L	Old L	Roots
1st week	–Se	n.d.	n.d.	n.d.	n.d.
	+Se	n.d.	n.d.	n.d.	n.d.
2nd week	–Se	52±5 [b]	77±3 [a]	53±5 [b]	18±1 [b]
	+Se	71±9 [a]	79±1 [a]	118±9 [a]	29±5 [a]
3rd week	–Se	n.d.	n.d.	n.d.	n.d.
	+Se	n.d.	n.d.	n.d.	n.d.
			Bolting		
1st week	–Se	72±11 [a]	65±3 [b]	65±3 [b]	36±8 [a]
	+Se	91±18 [a]	95±9 [a]	79±4 [a]	42±14 [a]
2nd week	–Se	80±11 [a]	93±8 [a]	82±7 [b]	25±3 [a]
	+Se	111±15 [a]	107±9 [a]	106±9 [a]	26±6 [a]
3rd week	–Se	78±7 [a]	69±15 [a]	80±8 [a]	36±6 [a]
	+Se	80±8 [a]	88±9 [a]	103±9 [a]	46±14 [a]
			Fruiting		
1st week	–Se	68±8 [a]	75±6 [a]	69±8 [a]	24±5 [a]
	+Se	68±9 [a]	68±8 [a]	64±9 [a]	28±9 [a]
2nd week	–Se	65±6 [a]	51±2 [a]	58±5 [a]	11±2 [a]
	+Se	50±1 [b]	54±6 [a]	57±3 [a]	16±5 [a]
3rd week	–Se	77±7 [a]	76±8 [a]	86±8 [a]	23±9 [a]
	+Se	88±9 [a]	80±9 [a]	79±1 [a]	12±1 [b]

Table 3: Concentration of soluble proteins (mg g^{-1} DW) in different shoot fractions and roots as influenced by the developmental stage of plants in the absence (−Se) or presence of Se (+Se) during three subsequent weeks in oilseed rape plants. Different letters between two Se treatments indicate statistically significant difference (*t*-test, $p \leq 0.05$). n.d., not determined

Harvest	Treatment	Rosette			
		Young L	Middle-aged L	Old L	Roots
1st week	–Se	n.d.	n.d.	n.d.	n.d.
	+Se	n.d.	n.d.	n.d.	n.d.
2nd week	–Se	27.6±2.2 [a]	15.8±1.2 [a]	16.2±3.8 [b]	4.6±0.6 [b]
	+Se	29.2±2.8 [a]	17.6±0.7 [a]	35.5±1.3 [a]	9.1±2.4 [a]
3rd week	–Se	n.d.	n.d.	n.d.	n.d.
	+Se	n.d.	n.d.	n.d.	n.d.
			Bolting		
1st week	–Se	34.8±1.3 [a]	15.9±2.1 [b]	9.45±0.9 [b]	9.3±0.7 [a]
	+Se	33.1±1.9 [a]	24.6±3.3 [a]	21.2±1.4 [a]	5.7±0.4 [b]
2nd week	–Se	34.9±1.8 [a]	19.5±1.3 [a]	12.9±1.4 [b]	8.7±0.7 [a]
	+Se	33.1±1.1 [a]	24.3±4.7 [a]	17.9±1.9 [a]	6.5±1.5 [a]
3rd week	–Se	34.2±1.1 [a]	11.9±0.9 [b]	5.25±0.9 [b]	8.0±1.2 [a]
	+Se	32.3±1.2 [a]	21.8±0.8 [a]	11.5±1.3 [a]	6.3±0.7 [b]
			Fruiting		
1st week	–Se	24.5±3.9 [a]	16.9±2.3 [a]	11.1±1.7 [a]	3.9±0.3 [a]
	+Se	22.9±2.6 [a]	14.6±4.9 [a]	10.3±0.6 [a]	4.5±0.4 [a]
2nd week	–Se	32.1±2.5 [a]	20.2±1.6 [a]	13.8±1.0 [a]	2.4±1.4 [b]
	+Se	29.1±4.6 [a]	19.0±2.1 [a]	12.3±1.2 [a]	4.2±0.3 [a]
3rd week	–Se	28.7±3.2 [a]	16.9±4.9 [a]	12.7±1.6 [a]	1.4±0.4 [b]
	+Se	23.7±1.7 [b]	19.9±2.8 [a]	10.8±1.1 [a]	2.6±0.2 [a]

Table 4: Allocation of nitrogen (% relative to total plant N) into different shoot fractions and roots as influenced by the developmental stage of plants in the absence (−Se) or presence of Se (+Se) during three subsequent weeks in oilseed rape plants. Different letters between two Se treatments indicate statistically significant difference (*t*-test, $p \leq 0.05$)

Harvest	Treatment	Rosette			
		Young L	Middle-aged L	Old L	Roots
1st week	–Se	37.8±1.67 a	31.0±3.50 a	9.60±0.96 b	21.6±1.26 a
	+Se	31.9±2.59 b	25.3±4.19 a	26.2±0.24 a	16.6±2.67 b
2nd week	–Se	44.3±4.18 a	34.6±2.18 a	10.8±3.22 b	10.3±2.57 a
	+Se	27.8±3.73 b	35.3±2.12 a	23.7±2.00 a	13.2±1.43 a
3rd week	–Se	43.6±5.30 a	33.8±6.30 a	12.2±4.38 b	10.4±3.49 a
	+Se	33.9±4.77 b	29.4±4.94 a	20.8±2.69 a	15.9±3.23 a
		Bolting			
1st week	–Se	42.1±1.93 a	26.8±3.33 a	16.6±1.14 b	14.5±1.47 a
	+Se	32.4±3.36 b	28.1±0.72 a	22.4±2.09 a	17.1±2.14 a
2nd week	–Se	43.3±9.48 a	27.3±3.05 a	11.4±2.70 b	18.0±2.88 a
	+Se	40.3±3.29 a	22.9±3.08 a	20.9±2.32 a	15.9±0.51 a
3rd week	–Se	35.9±7.48 a	24.4±1.31 a	22.7±2.73 b	17.0±2.48 a
	+Se	32.2±11.2 a	21.8±2.92 a	31.7±1.46 a	14.3±3.13 a
		Fruiting			
1st week	–Se	41.8±3.69 a	25.7±2.66 a	17.4±3.49 b	15.1±1.86 a
	+Se	38.6±2.14 a	27.0±3.80 a	21.9±0.77 a	12.5±4.56 a
2nd week	–Se	42.4±3.95 a	25.2±4.37 a	21.2±3.55 a	11.2±1.27 a
	+Se	38.5±4.16 a	26.3±5.62 a	24.8±2.09 a	10.4±3.91 a
3rd week	–Se	40.2±2.92 a	26.7±3.13 a	20.9±1.02 a	12.2±0.87 a
	+Se	38.0±2.37 a	28.4±3.28 a	22.9±3.38 a	10.7±1.15 a

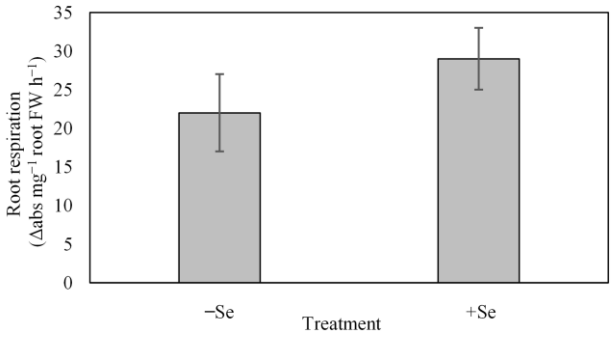

Fig. 5: Respiration rate measured by *triphenyltetrazolium chloride* (TTC) test in the roots of oil seed rape plantsat rosette stage in the absence (–Se) or presence of Se (+Se)

A significant interaction effect of developmental stage and harvest time was observed for all analysed parameters except for root DW, while interaction effects of Se treatment with other two parameters on the leaf photosynthesis rate were not significant indicating that Se influenced leaf photosynthesis consistently throughout the plant growth period (Table 5).

Discussion

Dry matter production was stimulated by Se only when plants harvested at fruiting stage (BBCH 73–88), while in plants harvested at bolting stage (BBCH 32–65), shoot dry mass was rather diminished by Se. In a field experiment with oilseed rape, the growth promoting effect of Se was observed from BBCH 63 onwards (Ebrahimi *et al.,* 2015). Lack of biomass increment at rosette stage and reduction of that at bolting stage in +Se plants, were observed, despite consistently higher rate of photosynthesis (up to 96%) in the leaves of different age. In addition, the biomass increment by Se at fruiting stage (2–15%) was much less than that of photosynthesis (8–57%). Carbon (C) fixed through photosynthesis is partitioned into metabolic, structural or storage pools (Kölling *et al.,* 2015). Here it seems likely that higher C partitioning into respiration, i.e., higher respiratory loss of fixed C, observed in +Se plants was the main reason for a negligible response to Se of biomass. In addition, different response of biomass to Se at bolting and fruiting stages implied that partitioning of the supplementary pool of fixed C in +Se plants into biomass production, metabolite synthesis and respiration was strongly dependent on the plant's developmental stage. Although the processes of photosynthesis and central C metabolism are quite well understood, little is known about the partitioning of C resources and its regulation. In order to investigate which metabolic processes preferentially use assimilated C, a ^{14}C labelling experiment was recently undertaken with Arabidopsis and revealed that partitioning of available C differs depending on leaf age and developmental status (Kölling *et al.,* 2015).

Leaf photosynthesis rate (μmol CO_2 m^{-2} s^{-1}) was steadily decreased during three subsequent harvests at rosette and bolting stages, being more prominent in the time between 1st and 2nd harvests at both stages. One of the earliest features of leaf senescence is reduction of photosynthesis concomitant with loss of leaf chlorophyll (Thomas, 2013; Gregersen *et al.,* 2013). Chloroplasts are the first organelles to deteriorate during senescence and the initial steps of chlorophyll and chloroplastic protein degradation are likely to take place within the plastid itself (Martínez *et al.,* 2008). At fruiting stage, however, photosynthesis rate increased between 2nd and 3rd harvest. Although silique wall and even developing embryos in oilseed rape are photosynthetically active organs (Hua *et al.,* 2012), leaves have higher photosynthesis rate than pods (Dreccer *et al.,* 2000) and thus are more effective in providing fruits with photosynthates. To the best of our knowledge, a comparative analysis on the leaf photosynthesis during different developmental stages in oilseed rape has not been reported so far, but a similar pattern as an inverted bell curve has been found in the leaves of olive (*Olea europaea* L.) after flowering (Proietti, 2001).

Effect of Se on promotion of net CO_2 assimilation rate observed in this work is in agreement with our previous findings with various species, in that it has been attributed to elevation of stomatal conductance and stimulation of photochemical events (Hajiboland and

Table 5: Results of three or two way ANOVA analyses with factors including developmental stage (D), harvest time (H) and Se treatment (S) and their interactions. Data of the leaves of different age including young leaves (YL), middle-aged leaves (ML) and old leaves (OL) were analyzed separately. Effects are presented as ns: non-significant and significant difference: *** P<0.001, ** P<0.01, * P<0.05

	D	H	S	D×H	D×S	H×S	D×H×S
Shoot DW	<0.001***	0.365ns	0.943ns	<0.001***	<0.001***	0.216ns	0.065ns
Root DW	<0.001***	0.002**	<0.001***	0.164ns	0.001***	0.633ns	0.987ns
Photosynthesis (YL)	0.006**	<0.001***	<0.001***	<0.001***	0.589 ns	0.832ns	0.361ns
Photosynthesis (ML)	0.082ns	<0.001***	<0.001***	<0.001***	0.502 ns	0.626ns	0.009**
Photosynthesis (OL)	0.002**	<0.001***	<0.001***	0.008**	0.127ns	0.860ns	0.002**
Respiration (YL)	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***
Respiration (ML)	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	0.096ns
Respiration (OL)	0.844ns	0.022*	<0.001***	<0.001***	0.233ns	0.830ns	<0.001***
Leaf SPAD (YL)	<0.001***	---	<0.001***	---	<0.001***	---	---
Leaf SPAD (ML)	<0.001***	---	0.149ns	---	0.733ns	---	---
Leaf SPAD (OL)	<0.001***	---	<0.001***	---	<0.001***	---	---
Carbohydrates (YL)	<0.001***	---	0.001***	---	<0.001***	---	---
Carbohydrates (ML)	<0.001***	---	0.017*	---	0.298ns	---	---
Carbohydrates (OL)	<0.001***	---	<0.001***	---	<0.001***	---	---
Proteins (YL)	0.014*	---	0.408	---	0.104ns	---	---
Proteins (ML)	<0.001***	---	0.018*	---	0.257ns	---	---
Proteins (OL)	<0.001***	---	<0.001***	---	<0.001***	---	---
N allocation (YL)	0.005**	0.024*	<0.001***	0.004**	0. 002**	0.337ns	0.183ns
N allocation (ML)	<0.001***	0.203ns	0.560 ns	0.009**	0.011*	0.793ns	0.020*
N allocation (OL)	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	0.004**	0.002**

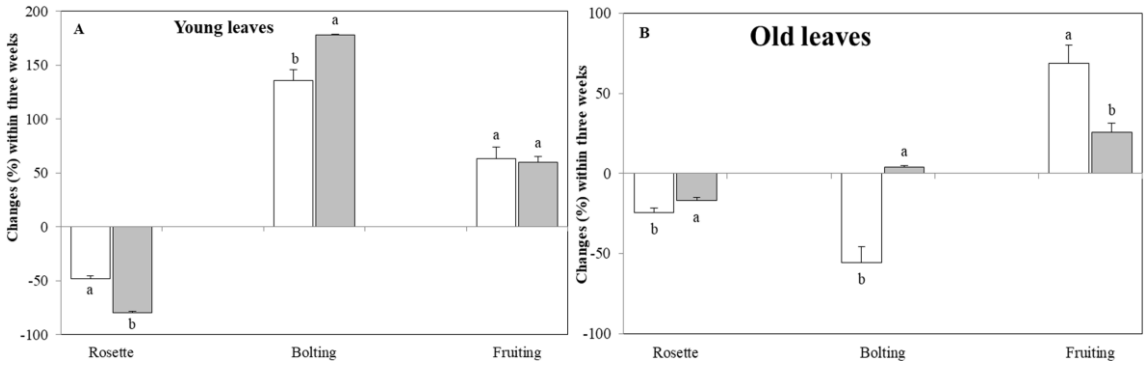

Fig. 6: Change of nitrogen content (% relative to 1st harvest) in the young (A) and old (B) leaves within three subsequent weeks in the absence (−Se) or presence of Se (+Se) at three developmental stages in oil seed rape plants

Keivanfar, 2012; Hajiboland *et al.,* 2014, 2015). Effect of Se on the upregulation of photosynthetic enzymes is another probable mechanism, as has been reported previously for Rubisco in apple leaves (Ning *et al.,* 2013). Se-mediated elevation of photosynthesis rate in the oilseed rape plants of this work was observed particularly in the old leaves, which could be at least partly linked to maintenance of photochemical activity in the old leaves in spite of beginning ageing processes. We have previously observed that Se delays leaf senescence via protection of leaf photochemistry from excessive radiation, particularly in the old leaves (Rahmat *et al.,* 2017).

Leaf respiration rate increased as expected during the time between 1st and 2nd week (except the middle-aged and old leaves at fruiting stage), which may be an indication of higher catabolising activity versus synthesis due to the progression of ageing in the leaves. The subsequent reduction of leaf respiration or its stable amounts observed after an initial increase in the leaves at rosette and bolting stages may indicate in turn a down regulation of catabolising activity of leaves during developmental senescence. In a study on *Arabidopsis* it was also shown that respiratory metabolism in leaves is strongly dependent upon leaf developmental stage (Markelz *et al.,* 2014).

Selenium treatment consistently increased respiration to different extents in the leaves of different age and at all three developmental stages. Root respiration in plants at rosette stage was also slightly higher in Se-treated (29 ± 5) compared with −Se (22 ± 4) plants. Stimulation of leaf respiration by Se has been reported previously for pea (Smrkolj *et al.,* 2006) and mung bean (Malik *et al.,* 2011), and was attributed to upregulation of enzymes related to starch and sucrose catabolism leading to elevation of sugars and subsequent increase in cellular respiration (Malik *et al.,* 2011). It has been stated that dark respiration is an important

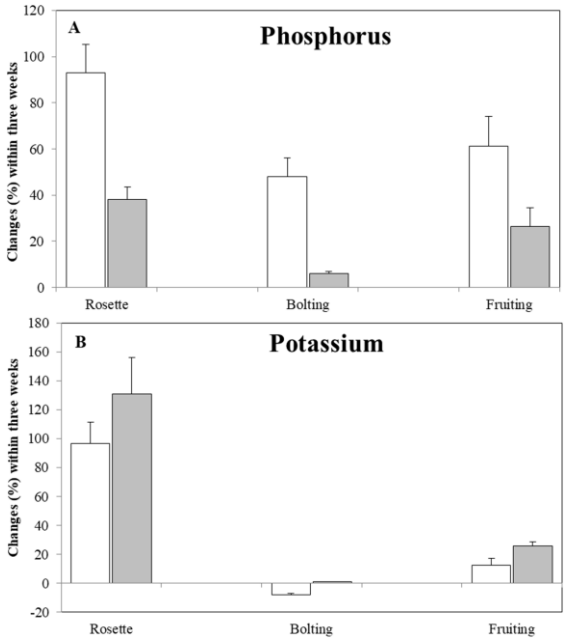

Fig. 7: Change of phosphorus (A) and potassium (B) content (% relative to that at 1st harvest) in the old leaves of oil seed rape plants within three subsequent weeks as influenced by plants developmental stage in the absence (–Se) or presence of Se (+Se)

part of the plant carbon budget (Valentini *et al.,* 2000). In the oilseed rape plants of this work, despite prominently higher photosynthesis rate in +Se plants, the concomitant increase of respiration may have prevented C partitioning into growth and was likely the reason for comparatively less improvement of biomass in Se-treated plants.

Concentration of soluble carbohydrates was mainly higher in +Se plants except in plants at fruiting stage. As was frequently observed in our previous works (Hajiboland *et al.,* 2014, 2015), accumulation of soluble sugars is expected from higher photosynthesis rate in the leaves upon Se treatment. Se-treated plants have higher sucrose synthase, sucrose phosphate synthase (Malik *et al.,* 2011) and fructose 1,6-bisphosphatase (Owusu-Sekyere *et al.,* 2013) activity. At fruiting stage despite a significant effect of Se on leaf photosynthesis, the soluble carbohydrates pool did not increase while greater dry matter was accumulated under these conditions. It may indicate higher C partitioning into biomass production than metabolite synthesis at fruiting, in contrast to that at rosette and bolting stages.

Effect of Se on the leaf soluble protein pool was strongly dependent on leaf age and plant developmental stage and, similarly with soluble carbohydrates, Se-mediated increase in protein concentration was particularly observed in the old leaves, except at fruiting stage. Higher activity of nitrate reductase in +Se plants was found in the young but not in the old leaves, both at rosette stage (Rahmat *et al.,* 2017), while its effect at further developmental stages has not been studied. Regardless of the mechanism, leaf protein content is an ultimate consequence of two processes both are influenced by Se, e.g., nitrate assimilation and/or proteins degradation.

Allocation of N to different shoot fractions was significantly influenced by Se depending on leaf age and developmental stage of plants. The most prominent effect was a significantly higher N allocation to the old leaves of +Se compared with −Se plants, in contrast to the young leaves at rosette and bolting stages. At fruiting stage, the effect of Se was observed only at first harvest.

In this work, decrease in the N content (% change) during three subsequent weeks revealed that at rosette stage, N remobilisation occurred not only in the old but also in the young leaves. Physiological study of N remobilisation during sequential leaf senescence in *B. napus* revealed that N remobilisation occurs precociously when leaf maturity has been attained and before the real commencement of senescence processes (Avice and Etienne, 2014). In the oilseed rape plants of this work, the young leaves were more efficient in the remobilisation of N than were the old leaves (Fig. 6). Based on a weekly ^{15}N-labelling in field conditions during the oilseed rape growth cycle, Malagoli *et al.* (2005) showed that the leaves in the upper canopy were more efficient in terms of N mobilisation than were the lower leaf ranks.

In relation to the effect of Se on the extent of N remobilisation, a considerable difference between the young and old leaves was observed at rosette and bolting stages. Acceleration of N remobilisation as judged by higher reduction of N content in +Se plants was observed in the young leaves while the opposite was found in the old leaves at rosette and bolting stages.

At fruiting stage, both young and old leaves failed to remobilise N and instead, acted as sink for root-derived N. In the old leaves of +Se plants, however an obvious N depletion compared with −Se plants occurred; thus, old leaves may act as a source of N for developing fruits and reduction of N in favour of reproductive organs may be the mechanism for up to 2.2-fold higher fruit yield observed in +Se plants previously (Hajiboland and Keivanfar, 2012; Ebrahimi *et al.,* 2015). Oilseed rape has a low N-use efficiency, mainly due to the low N remobilisation efficiency observed during the vegetative phase when sequential leaf senescence occurs (Avice and Etienne, 2014). Our results suggest that application of Se to field-grown oilseed rape may significantly enhance N-use efficiency in this crop.

Interestingly, percentage of change of P and K did not follow the pattern observed for N as related either to the leaf age and plant developmental stage or to the effect of Se. It suggests a particular role of N remobilisation and homeostasis in determination not only of developmental senescence but also of its delay mediated by Se.

Carbon-to-nitrogen ratio appears to play a

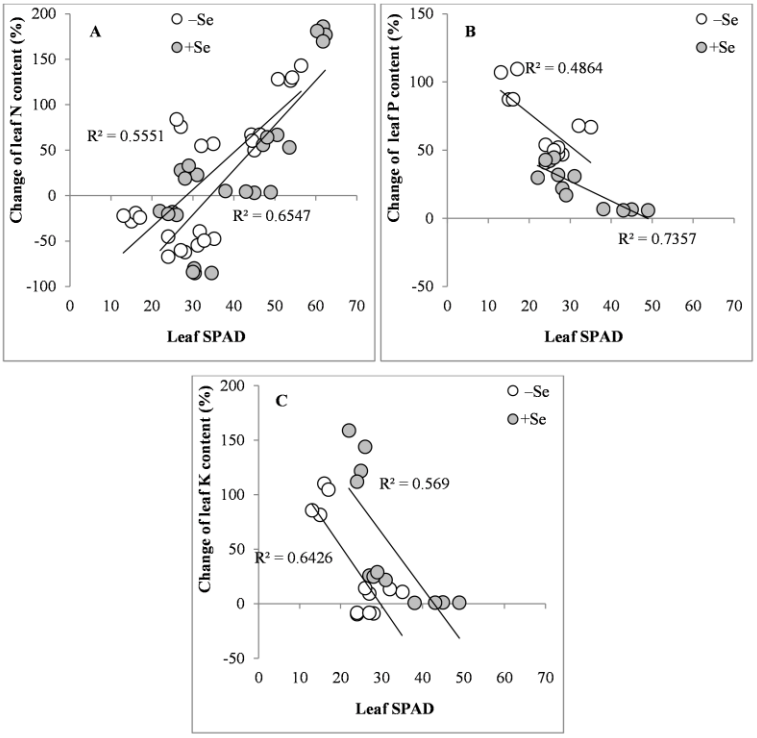

Fig. 8: Correlation between leaf SPAD and % change of leaf nitrogen (A), phosphorus (B) and potassium (C) in the absence (−Se) or presence of Se (+Se) in oil seed rape plants

predominant role in regulating various aspects of plant growth, including storage reserve mobilisation, photosynthetic gene expression (Martin *et al.*, 2002) and senescence (Palenchar *et al.*, 2004). Study of C and N partitioning and allocation may help to elucidate the mechanism of Se effect on delaying leaf senescence independent from its antioxidative effects. Our results demonstrated that the senescence delaying effect of Se at rosette and bolting stages observed in this work was accompanied by a significant rise of the soluble C pools in the old leaves and was paralleled with an increase of N compounds. Even at fruiting stage, characterised by the lack of Se effect on senescence of leaves, stable amounts of both C and N metabolites prevents again an imbalance in the ratio of carbohydrates to nitrogenous compounds.

Conclusion

Our results suggest that Se enhances the green-leaf area duration and thus, may increase the potential of oilseed rape plants to raise yield under field conditions and may contribute significantly to continued N uptake, that is of particular importance for this species with low N efficiency.

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