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EFFECT OF DEFOLIATION AND SILIQUE REMOVAL ON CARBOHYDRATES ACCUMULATION, SEED QUALITY, AND YIELD OF RAPESEED (*BRASSICA NAPUS* L.)

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Abstract

Rapeseed is one of the important oil seed crop and it has many studies related to its yield and oil quality. There is a lack of quantitative information on the effect of rapeseed yield and quality by management measures (Defoliation and silique removal). This study was designed to investigate defoliation and silique removal treatments effect on the physiological process at the flowering stage, the quality and quantity of crops. A field trial was carried out at the Experimental Research Station of Sichuan Agricultural University in Sichuan province of China, in 2020. The split-plot field experiment was designed with treatments as the main plot and variety as a subplot with three replicates. The leaf and silique removal treatments were applied to investigate their seed yield and quality behaviors at the flowering stage. This experiment was conducted by using two high-yielding varieties, V_1=JYJS01: medium height variety and V_2=CY36: tall height variety to investigate the impact of different leaf and silique removal treatments, T_1=control; T_2=50% branch silique removal; T_3=50% defoliation; T_4=100% branch silique removal and T_5=100% defoliation. Our results have revealed that the effect of defoliation and silique removal treatments affected the N uptake of rapeseed plants. During the physiological maturity stage of rapeseed, treatment T_4 significantly amplified the protein content by 9%, while the seed oil content was decreased by 3.95% compared to T_1. Moreover, in contrast, the stem sucrose and reducing sugar content were decreased at T4 by 26.70% and 42.80 % compared to T_1, respectively. The seed yield and plant biomass was also decreased at T_4 compared to T_1. Our findings revealed that higher stem nitrogen percentage and sugars metabolism was possible during the seed filling stage of rapeseed by defoliation and silique removal treatments. Further research is required to investigate the involvement of molecular mechanisms in the fatty acid metabolism of rapeseed.

Key words: Rapeseed (*Brassica napus*. L); Carbohydrates accumulation; Yield; Seed quality.

Introduction

Crop production depends upon physiological processes such as photosynthesis, photoassimilates transportation, and active sinks formation. Green organs (leaves), essential for photosynthesis, play their role as a source. In contrast, seeds are important sinks. There are numerous elements which affect the relationship between the source and the sink. For instance, nitrogen (N) and carbon (C) play their significant role in photosynthesis and in defining the source-sink potential and, ultimately, the seed yield and quality. The source's function determines the sink's metabolic processes and is associated with the optimized usage of C and N (Burnett, 2019). Seeds act as a sink, and they are provided with assimilates of nearby leaves. As plants grow further, new leaves form, and the function of photosynthesis decreases (Doğru & Çakirlar, 2020). Leaves determine the photosynthetic potential and considerably impact yielding behavior (Zhang & Flottmann, 2018). The inclusion of the canopy (above-ground plant part) architecture influences the light interception capacity of each leaf (Beadle *et al*., 1985). Light interception declines top to bottom within the aerial part of the plant. Henceforth, lower leaves are photosynthetically weaker than the upper leaves. Thus, one feasible crop quality and quantity improvement strategy is maintaining adequate leaves areas for high photosynthetic efficiency and metabolic activities (Lone *et al*., 2008). A suitable leaves surface may be adjusted by removing some leaves and silique. Partially or full exclusion of leaves has been known as defoliation, an old age exercise in numerous world areas. It allows photosynthetically highly active new-grown leaves to grow fast and effectively utilize given water and essential nutrients (Khan *et al*., 2008).

Availability of C and N during the growth of the plants is necessary for the flower and silique setting (Lawlor, 2002). Leaf shading causes mobility of C and N distribution. A shortage of N affects Brassicaceae's morphology (Brunel-Muguet *et al*., 2013; Papantoniou *et al*., 2013; Khan *et al*., 2018), decreasing the growth rate, and increases the level of starch in the leaf which reduces the photosynthetic rate and RuBisCO activity. The process of photosynthesis disturbs due to alterations in the elements which are necessary for photosynthesis during the vegetative stage development of leaves (source) which is more than the reproductive organ (sink). Source to sink ratio is very important for assimilates' supply and demand, which interferes with photosynthesis. When the capacity of the sink (seed) enhances then assimilation demand in the silique increases, which requires high source strength for the growth and development of seeds. The flowering stage is critical because plants transport most of the assimilates towards the reproductive organs to develop seed volume (Faraji, 2014). During the flowering stage, shading reduces the seed number and increases the duration and rate of the seed-filling period (Labra *et al.*, 2017). Consequently, manipulating the source and sink relationship by defoliation and silique removal treatments causes changes in the plants' physiological processes (photosynthesis, respiration, electron transport chain), which ultimately affects the quality and quantity of the crops.

There are some stages in the life of the plants which are vital and have great importance in the source and sink relationship. Manipulation of source and sink capacity in these stages influences photosynthetic activity. For example, if silique are removed at the reproductive stage of plants, then sink capacity decreases, and source capacity increases due to having more assimilate level than supply to sink, resulting in the decline of ribulose-1,5-bisphosphate carboxylase (RuBisCO) which causes the stomatal closure. The stomatal closure results in the decline of the leaf photosynthesis (Brestic & Zivcak, 2013) by adjusting the source and sink capacity (by defoliation and silique removal treatments) that minimized the starch accumulation level in the leaves and maintains the photosynthesis, hence defoliation and silique removal treatments affects the physiological process (photosynthesis). The decrease in photosynthesis causes a reduction of assimilate availability (Zhang & Flottmann, 2018), which ultimately influences the quality and quantity of the crops. However, many studies have revealed the effect of defoliation and pod removal on the physiological mechanisms of different crops. However, their influence on underlying physiological regulations in rapeseed has not been elucidated yet.

Therefore, this study hypothesized that seed quality depends upon the photoassimilates availability and photoassimilates limitation in canola depends upon the growth stage, the flowering stage. Thus, in this study, assimilates availability was manipulated by defoliation and silique removal treatments at the flowering stage to investigate the physiological regulations in rapeseed seed quality.

Materials and Methods

Varieties: There are two types of rapeseed plant varieties used in this experiment: JYJS01, a medium height variety denoted by V1, and CY36, a tall height variety denoted by $V_{1.}$ The source of V_1 was the rapeseed research center, Sichuan, China. And source of V_2 was the Agricultural Academy of Sciences, China.

Trial design and site: The field trial was carried out at the Experimental Research Station of Sichuan Agricultural University in the higher parts of the Yellow River Basin of China in Sichuan (102˚54 E, 104˚53 E, 30˚05 N, 31˚26 N). The rainfall is 900-1300 mm/year, mean temperature remains 16˚C, and duration of sunshine light is 1041-1413 h. In the winter season, the daily mean precipitation is lower than 150 mm and daily temperature in December and February remains greater than 0˚C. (Fig. 1) illustrates the climatic conditions of the experimental site, which include the monthly rainfall and average temperature. The soil of the experiment site was tested, texture clay loam with 55% of sand, 27% of silt, and 18% of clay (determined by using the hydrometer method). Total nitrogen, hosphorous and potassium was 0.8 g kg^{-1}, 6.25 g kg^{-1} and 0.63 g kg^{-1}, respectively (Huoyan *et al*., 2016; Sparks *et al*., 2020). Available nitrogen was 63.34 mg kg^{-1} (Clever chem 200, Germany). Accessible potassium, available phosphorous, organic matter and pH of the upper soil layer (0-25 cm) were 95.28 mg kg^{-1} (Jackson, 2005), 40.61 mg kg^{-1}, 30.42 g kg^{-1} and 6.6 (Pietsch & Mabit, 2012), respectively.

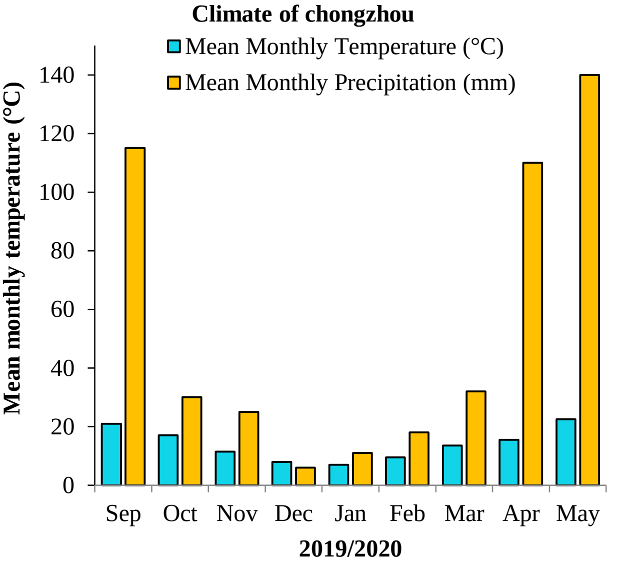

Fig. 1. Average monthly precipitation and the temperature from September to May in the growing season of 2019/20.

Field trial was executed during 2019-2020. Design of the experiment was split-plot, with the treatments as the main plot and variety as a subplot repeated three times. The subplot area was gauged as 9 × 2m. The entire ploting site was given 180kg N ha^{-1}, 90 kg P_2O_5 ha^{-1}, and 90 kg K_2O ha^{-1}. The Source of N was urea, P_2O_5 was $CaH_6O_9P_{2,}$ and K_2O was K_2SO_4. The entire N fertilizer dose was divided into two stages. The first time was given as a base before sowing. The second time was given manually by top-dressing at the vegetative (6-leaf stage) at the end of November. Other fertilizers were applied in the field before sowing. Sowing in the sub-plot was done by seeding three-kilogram seeds in one hectare. The distance between the rows was kept 30cm, and the space between the hill was 20cm. The crop was sown on 11th October, 2019. The flowering stage was started on 24^{th} February and ended on 24^{th} March 2020.

Measurements and methods: Plants of the same height, diameter, and branches at the initial flowering stage were individually selected, tagged for identification, and treated according to design. One row was selected out of 5 rows from each plot for (T_1) control treatment, one row for (T_2): 50% branch silique removal, in which 50% branch silique were removed while the main central branch silique remained intact, and one row for (T_3): 50% defoliation, in which 50% upper leaves were removed and the other lower leaves remained intact, and one row for (T_4): 100% branch silique removal: all the branches silique were removed except the main central branch. One row selected for (T_5): 100% defoliation, in which all the leaves (lower and upper part) were removed. All treatment applications are illustrated in (Fig. 2). For each treatment, 10 plants were selected from each row out of five. The leaves and silique were removed with the help of pruning scissors. At the maturity stage, 6 plants from each treatment of every replication were harvested for seed quality and yield determination. The plant samples were dried in the oven at 105°C for one hour for stopping enzymatic activities. Then they were retained at 80°C to attain a constant weight. Afterward, the samples biomass was determined by weighing balance.

Seed oil and protein: The seed oil and protein content were determined at the harvesting stage. These contents were determined using Near-infrared reflectance spectroscopy, NIRS; Foss NIR Systems Inc., USA by following the protocol of (Hu *et al*., 2009).

Nitrogen: The plant samples were dried in the oven at 105°C for one hour for stopping enzymatic activities. Then they were retained at 80°C to attain a constant weight. Afterward, the samples were ground into powder, and the nitrogen contents of the sampling plant parts were determined following the Kjeldahl procedures (Keeney, 1982; Sparks *et al*., 2020).

Sugars extractions: Fresh stems samples were taken to determine the sugar contents and dried in oven at 105°C for ca 30 minutes and kept 12 hours at 80°C until a constant dry weight. Then, 0.1g mashed powder of the sample was taken, and then 6 ml of eighty percent of ethanol was poured in it. Furthermore, we boiled the test material at 80°C for 40 min and then passed them through centrifugation (5000 rpm/5.min). Then the upper floating solution was poured into test tube (50 ml) of primary solution. This procedure was repeated twice. After that, solution (80% ethanol) was poured into the primary solution to make up 50ml volumes. And then, 0.1g of charcoal was poured to every 50 ml tube and retained for 12 hours until the greenish shade was disappeared. The resultant solution was purified by passing through the filter and used to determine sugars (Ghafoor *et al*., 2022).

Sucrose: To exmine the sucrose content, 0.9 ml of extracted solution was taken and poured into a 10 ml sample tube and then 0.1 ml of 2M NaOH was mixed and warmed up (about 10 minutes) by using a water bath. After warm up, samples were retained for about 12-15 minutes to decrease temperature to room temperature. After this, one milliliter and 3 milliliters of 0.1% resorcinol and 10M HCL were poured into the solution, respectively, and heated (80°C about 10 min). After this step, the absorbance of the supernatant at 480 nm was determined using the spectrophotometer (SpectraMax i3x from Austria).

Reducing Sugar: We mixed up three solutions to determine the reducing sugar in the 10 ml sample tube: 1.5 ml test solution, 1.5 ml DNS, and 0.5 ml dd H_2O. These tubes were warmed up in a water bath at 80°C for ten minutes. The absorbance of the resultant supernatant was obtained at 520 nm by using the spectrophotometer (SpectraMax i3x from Austria).

Statistics: Analysis of variance (ANOVA) and ' 'Duncan's test was utilized to know the statistically significant differences between the treatments and their combinations (p<0.05). IBM SPSS Statistics 21.0 was used for analyses.

Result

Seed protein content: The results indicated that seed quality parameters (seed protein and oil content) were significantly influenced by the interaction effects of defoliation and silique removal treatments (Table 1). Relative to T_1, the seed protein content increased by 7.95, 8.03, 10.26, and 8.03% at T_2, T_3, T_4, and T_5, respectively in V_1. Similarly, the seed protein content was increased by 6.72, 4.42, 7.80, and 7.48% at T_2, T_3, T_4, and T_5, respectively relative to T_1 in V_2. Overall, comparison with T_1 treatment, T_2, T_3, T_4, and T_5 significantly increased the seed protein content by 7.32, 6.18, 9, and 7.75%, respectively (Table 1). Generally, in all treatments and varieties the higher seed protein content was observed by treatment T_4 and variety V_2, and the trend was obtained as T_4>T_5>T_2>T_3>T_1 and V_2 performed better than V_1, respectively.

Seed oil content: The current findings of our experiment exhibited that the seed oil content was significantly changed by the interactive effects of defoliation and silique removal treatments (Table 1). In contrast with the seed protein, the seed oil content was decreased by 1.18%, 3.08%, 4.59%, and 4.27% at T_2, T_3, T_4, and T_5 compared with T_1, respectively in V_1. Moreover, the seed oil content also declined by 2.09%, 1.69%, 3.32%, and 3.32% at T_2, T_3, T_4, and T_5 compared with T_1, respectively in V_2. The seed oil content was also decreased in treatments T_4>T_5 >T_3 >T_2 >T_1 with 1.64%, 2.38%, 3.95%, and 3.79% at T_2, T_3, T_4, and T_5, respectively, compared to T_1. The seed oil content decreased by 4.62% in V_1 compared to V_2, respectively. However, V_1 and T_4 showed the lower seed oil content, and the total oil content was represented as T_4<T_5<T_3<T_2<T_1 and V_1<V_2, respectively.

Carbohydrates: The stem carbohydrates content (sucrose and reducing sugar) was decreased significantly by the interaction effects of defoliation and silique removal treatments (p<0.05.) (Table 2). The difference between the varieties was insignificant (p>0.05) for sucrose and reducing sugar overall. Our results showed that the stem sucrose and reducing sugar contents were decreased significantly at T_2, T_3, T_4, and T_5 compared to T_1.

Sucrose content: It has been observed that the stem sucrose content was reduced significantly by the defoliation and silique removal treatments (p<0.05.). Table 2. The stem sucrose content was decreased at T_2, T_3, T_4, and T_5 by 25.34, 24.80, 28.86, and 27.15% compared to T_1 in V_1. Furthermore, the same reduction was found in V_2, in which the stem sucrose content was decreased by 24.53, 18.53, 38.51, and 30.91% in V_2, as compared with T_1. In general, in all treatments, the sucrose content was decreased at T_2, T_3, T_4, and T_5 by 22.28, 18.47, 26.70, and 24.66 compared to T_1. On average, the stem sucrose content was decreased by 13.69 in V_2 compared to V_1. Moreover, low stem sucrose content was observed in treatment T_4 and variety V_2, and the trend was obtained as T_4<T_5<T_2<T_3<T_1 and V_2<V_1, respectively.

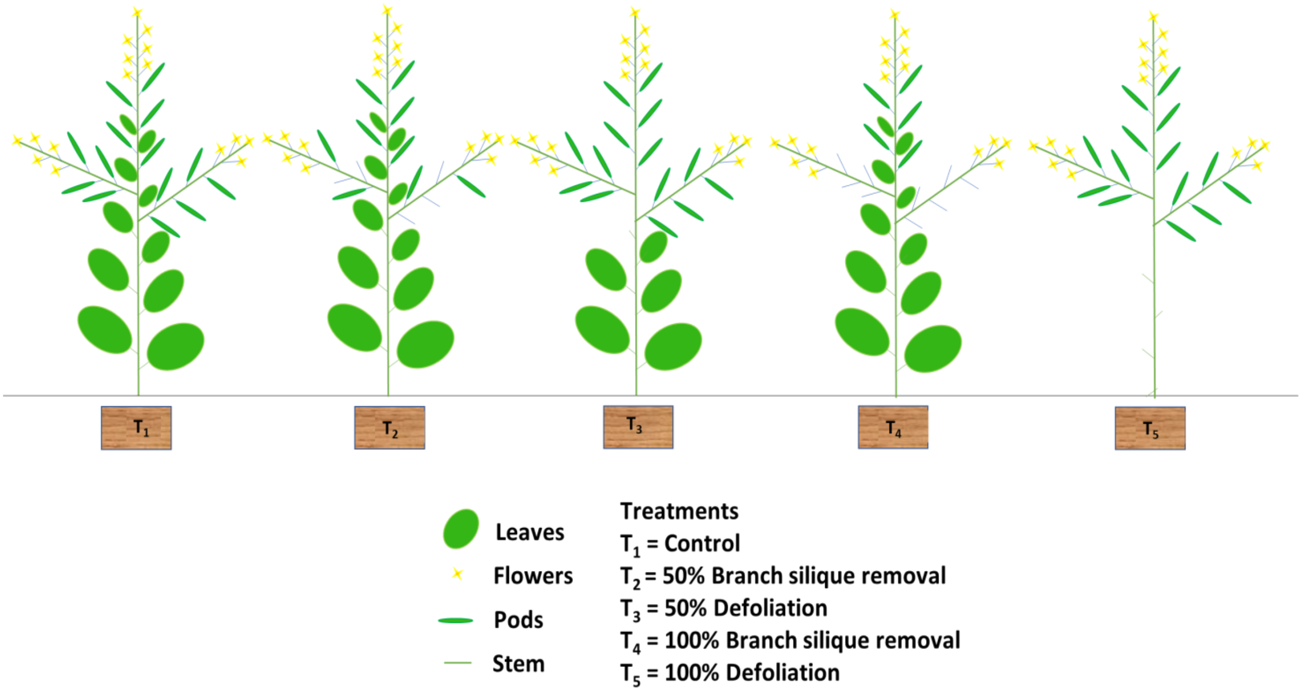

Fig. 2. This model diagram illustrates the treatments T_1 = Control; T_2 = 50% branch silique removal; T_3 = 50% defoliation; T_4 = 100% branch silique removal and T_5 = 100% defoliation.

Table 1. Effect of different treatments (T_1=control; T_2=50% branch silique removal; T_3=50% defoliation; T_4=100% branch silique removal and T_5=100% defoliation) on seed protein content, oil content, seed yield, stem N%, and seed N% of two varieties. The V_1 and V_2 represent the variety 1 and 2, respectively.

Variety	Treatment	Seed protein content (%)	Seed oil content (%)	Seed yield (g plant^{-1})	Biomass (g plant^{-1})	Stem nitrogen (%)	Seed N %
V_1	T_1	17.61b	47.34a	11.78a	37.30a	0.63c	2.82b
	T_2	19.01a	46.78ab	4.93bc	21.40b	0.63abc	3.04a
	T_3	19.02a	45.89bc	6.47bc	22.37b	0.60bc	3.04a
	T_4	19.41a	45.17c	4.43c	15.90c	0.66a	3.11a
	T_5	19.02a	45.32c	4.65c	17.85c	0.64ab	3.04a
V_2	T_1	18.46b	48.35a	12.41a	40.64a	0.60b	2.95b
	T_2	19.70a	47.34ab	7.39bc	29.17bc	0.65ab	3.15a
	T_3	19.27a	47.53ab	9.43b	32.35b	0.62b	3.09a
	T_4	19.90a	46.74bc	5.39c	19.79d	0.71a	3.19a
	T_5	19.84a	46.75bc	6.56c	24.01cd	0.67ab	3.17a
Treatment difference	T_1	18.03b	47.85a	12.10a	38.97a	0.59d	2.89b
	T_2	19.35a	47.06b	6.16c	25.29bc	0.64bc	3.10a
	T_3	19.15a	46.71bc	7.95b	27.36bc	0.61cd	3.06a
	T_4	19.66a	45.96c	4.81c	17.85d	0.69a	3.15a
	T_5	19.43a	46.03c	5.58c	20.93cd	0.66ab	3.11a
	T	***	***	***	***	***	***
	V	***	***	Ns	*	*	*

The same letter's columns denote a non-significant difference (p<0.05). The LSD test calculates it; as n=15. The ***, ** and * show the significant at p<0.001, 0.01 and 0.05, respectively. And ns show the insignificance

Table 2. Treatments (T_1=control; T_2=50% branch silique removal; T_3=50% defoliation; T_4=100% branch silique removal and T_5=100% defoliation) affect the stem sucrose and reducing sugar contents. The V_1 and V_2 represent the variety 1 and 2, respectively.

Variety	Treatment	Sucrose (mg/g)	Reducing sugar (mg/g)
V_1	T_1	6.653a	0.631a
	T_2	4.967a	0.513bc
	T_3	5.003a	0.549b
	T_4	4.733a	0.432d
	T_5	4.847a	0.466cd
V_2	T_1	5.420a	0.870a
	T_2	4.417bc	0.457a
	T_3	4.840ab	0.510a
	T_4	4.117c	0.427a
	T_5	4.250bc	0.473a
Treatment difference	T_1	6.037a	0.750a
	T_2	4.692b	0.485b
	T_3	4.922ab	0.529ab
	T_4	4.425b	0.429b
	T_5	4.548b	0.470b
	T	Ns	ns
	V	Ns	ns

The same letter's columns denote a non-significant difference (p<0.05). The LSD test calculates it; as n=15. The ***, ** and * show the significant at p<0.001, 0.01 and 0.05, respectively. And ns show the insignificance

Reducing sugar content: The reducing sugar content was decreased significantly by the interaction effects of defoliation and silique removal treatments (p<0.05.) Table 2. In this experiment, the reducing sugar content decreased at T_2, T_3, T_4 and T_5 by 18.66, 13.00, 31.50, and 26.07% in V_1 and 47.51, 41.38, 50.95, and 45.60% in V_2, respectively, compared to T_1. Overall, the stem reducing sugars content was reduced at T_2, T_3, T_4 and T_5 by 35.33, 29.47, 42.80, and 37.33 compared to T_1, respectively. Moreover, in all varieties, the stem-reducing sugar content was decreased by 14.66 V_1 compared with V_2**.** The lower stem-reducing sugar content was observed by treatment T_4 and variety V_3, and the trend was obtained as $T_4<T_5<T_2<T_3<T_1$ and $V_1<V_2$, respectively.

Stem nitrogen percentage: Defoliation and silique removal treatments showed a significant increment in stem and seed nitrogen percentage (p<0.05.) Table 1. It was noticed that defoliation and silique removal treatments increased the stem nitrogen percentage at T_2, T_3, T_4 and T_5 by 7.44, 3.43, 13.15, and 9.72% in V_1, compared to T_1. Moreover, in variety V_2, the nitrogen percentage was also increased by 7.78, 3.30, 18.45, and 12.22% compared to T_1. Overall, in all treatments, the stem nitrogen percentage was increased by 7.60, 3.38, 15.71, and 10.98% at T_2, T_3, T_4 and T_5 compared to T_1, respectively (Table 1). Low stem nitrogen percentage was observed in variety V_1. Overall, the maximum stem nitrogen percentage was observed in treatment T_4 and variety V_2, and the trend was obtained as $T_4>T_5>T_2>T_3>T_1$ and $V_2>V_1$, respectively.

Seed nitrogen percentage: The current findings exhibited that the applied treatments (defoliation and silique removal) significantly affected the seed nitrogen percentage (Table 1). It was found that the stem nitrogen percentage was increased at T_2, T_3, T_4 and T_5 by 7.80, 7.80, 10.28, and 7.80 as compared to T_1 in V_1, Furthermore, the same increasing trend was found in V_2 in which the seed nitrogen percentage was increased by 6.78, 4.75, 8.14 and 7.46% in V_2, as compared with T_1. In general, according to all treatments, the seed nitrogen percentage was increased at T_2, T_3, T_4 and T_5 by 7.27, 5.88, 9, and 7.71 compared to T_1, respectively. Moreover, the higher seed nitrogen percentage was observed by treatment T_4 and variety V_2, and the trend was obtained as $T_4>T_5>T_2>T_3>T_1$ and $V_2>V_1$, respectively.

Seed yield and biomass: The current findings evaluated the effect of different defoliation and silique removal treatments on rapeseed cultivar seed yield and biomass. As exhibited (Table 1.), the minimum seed yield (4.43 g plant-1) was recorded at T_4 in V_1 compared to T_1 in both varieties. Furthermore, the minimum seed yield was recorded in V_1(4.43 g plant^{-1}) ccompared to V_2(5.39 g plant^{-1}) at T_4. The plant biomass was minimum at T_4 compared to other treatments. The minimum plant biomass of both varieties was recorded in treatment T_4 (17.85 g plant^{-1}), whereas the maximum plant biomass (12.10 g plant^{-1}) was recorded in T_1. The varietal difference in seed yield was not significant. In contrast, the biomass difference between the varieties was substantial.

Correlation analysis: As represented in figure 3, the stem nitrogen percentage had significantly negative correlations with stem carbohydrates (sucrose and reducing sugar) and seed oil content. However, there were significant (p≤0.05) positive correlations between the rapeseed stem nitrogen percentage and seed protein content (Fig. 3).

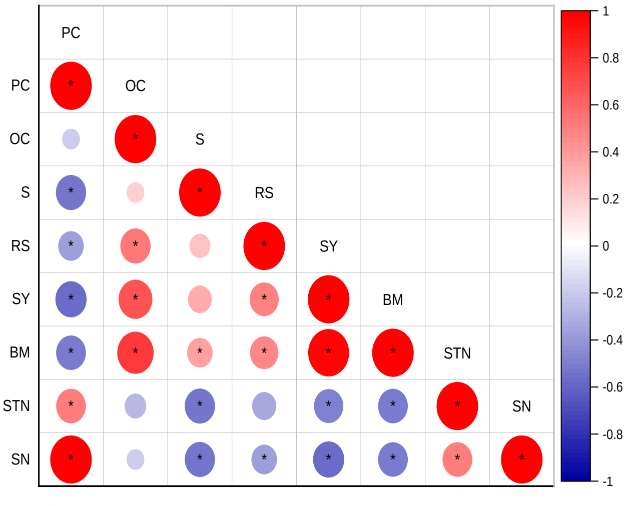

Fig. 3. Correlation analysis of seed protein content (PC), oil content (OC), stem sucrose content (S), stem reducing sugar content (RS), seed yield (SY), biomass (BM), stem nitrogen percentage (SN), and seed nitrogen percentage (SN). The circle size represents the correlation's significance, and the circle's color shows the positive or negative correlation. The dark red represents the more significant positive correlation, and the dark blue color represents the more significant negative correlation. The statistically significant difference between the treatments was illustrated by * at probability level p≤0.05.

Discussion

Crop production depends upon physiological processes such as photosynthesis, photoassimilates transportation, and active sinks formation (Ainsworth & Bush, 2011). Photosynthetic green organs (leaves), machinery for photoassimilates formation, play their role as source whereas seeds are considered one of the important sinks (Zhang & Flottmann, 2018). Many factors are affecting the assimilates availability. For instance, the photosynthetic efficiency and relationship between nitrogen (N) and carbohydrates (C) play a significant role in defining the assimilates availability and seed quality (Cai *et al*., 2020). Any change in plant canopy (above-ground plant part) improved the N utilization efficiency of crop plants (Raza *et al*., 2020). In present experiment, defoliation and silique removal treatments altered seed yield, plant biomass, stem, and seed nitrogen percentages, seed protein, and oil concentration.

Moreover, the defoliation and silique removal treatments decreased the seed yield and plant biomass, while improving the nitrogen uptake; however the nitrogen uptake altered the seed protein and oil concentration. The current findings were in agreement with the previous studies in which it was demonstrated that defoliation decreased seed yield and biomass accumulation (Barimavandi *et al*., 2010; Alimohammadi & Azizov, 2019). Moreover, treatments (defoliation and silique removal) profoundly affected the nitrogen uptake and carbohydrate's metabolism, which influenced the seed protein and oil concentration. Photoassimilates synthesized in the leaf are utilized and transported to maintain the plant and develop new organs, stems, bracts, flowers, silique, and seeds (Patrick & Colyvas, 2014). Contrary to the understanding that reproductive tissues act only as resource sinks, it was reported before that silique were photosynthetically active (Raven & Griffiths, 2015; Brazel & Ó'Maoiléidigh, 2019; Li *et al*., 2019). The photosynthetic efficiency of carbon and nitrogen metabolic activities is an integrated and synchronized phenomenon (Noctor & Foyer, 2000; Khan *et al*., 2008).

Silique and leaf removal treatments altered the N uptake pattern, which increased photosynthetic efficiency which was linked with the increase in nitrogen assimilation. Stockhoff, (1994) and Iqbal *et al*., (2012) reported that defoliation losses are compensated by increased irradiance of leaves after defoliation and N remobilization. Moreover, it was also suggested by Iqbal *et al*., 2012, that defoliation was one of the vital strategies for improving crop plant's N utilization efficiency. Previous research revealed that defoliation increased the N uptake (Liu *et al*., 2015). However, in this experiment, the treatment T_4 has considerably increased nitrogen accumulation. Regarding this finding, it was previously confirmed that the availability of light delayed the process of leaf falling (senescence) (Liu *et al*., 2015) and hence enhanced the photoassimilates availability, which enhanced the nitrogen accumulation (Liu *et al*., 2015). However, our findings indicated that treatment T_4 proliferated the N uptake during the silique filling stage, which reduced the stem carbohydrates (sucrose and reducing sugar) content, ultimately affecting the carbohydrates metabolism in the seed, hence altering the seed protein and oil concentration.

Defoliation causes alterations in the availability of photoassimilates and may affect carbon (C) portioning. Transportation of C in leaves is considered to be linked with photosynthetic apparatus in different plant species such as sugar beet (*Beta vulgaris* L.) (Servaites *et al*., 1989), cotton (*Gossypium hirsutum* L.) (Hendrix & Huber, 1986), sorghum *(Sorghum bicolor* L.) (Wardlaw, 1990). The responsive phenomenon of plants to low C availability through leaf and silique removal treatments usually enhances assimilates' transportation to shoot growth than root growth (Yang & Midmore, 2004). It was reported from previous studies that silique removal and defoliation needed high energy stock (Reichman & Smith, 1991), causing the plants to reallocate (remobilizes stored nitrogen) the energy stored in remaining leaves and silique skin, shoots, and roots for compensating its energy requirements (Liu *et al*., 2007). It was reported in the Raza's findings that defoliation at vegetative stage improved the nutrients uptake from the soil (Raza *et al*., 2019). According to previous studies, it was known that silique removal and defoliation in rapeseed had increased the nitrogen accumulation to meet the stem nitrogen demand (Lone & Khan, 2007). However, in our experiment treatment, T_4 increased the stem nitrogen accumulation to make it available for seed metabolism. Ni, *et al*., (2019), observed that nitrogen effectively inhibited sugar production and led to a diluting effect on the oil concentration. Nitrogen's role in seed quality improvement needs further investigation. It was reported earlier that nitrogen decreased carbohydrate accumulation deposition (Mokhtassi-Bidgoli *et al*., 2013; Pavithra *et al*., 2014). In our current findings, silique removal and defoliation treatments amplified the nitrogen accumulation (high stem and seed N percentages) and decreased the stem sugar accumulation (Fig. 4).

Moreover, higher nitrogen concentration reduced the carbohydrates translocation for seed metabolism. Besides, nitrogen is important in rapeseed seed quality and the accumulation of sugars (sucrose and reducing sugars). Carbohydrates (Sugars) are very important when studying seed physiological processes. And they can shift their location from stem to seed and play their essential role in seed physiological functions. In seeds, sugars are the main and very important source of C for fatty acid metabolisms, which leads to the seed's effectiveness. The C:N ratio was higher, and oil concentration was lower in the silique wall under the control treatment (N was not applied) (Ni *et al*., 2019). According to our trial experiment, silique and leaf removal treatments had declined the stem sucrose and reducing sugar content due to the reallocation of reserve N from other plant parts towards the stem. The photoassimilates i.e., sucrose, perform a vital function in the construction of fatty acids because they can be converted into fatty acids during lipid biosynthesis (Zhang & Flottmann, 2018). Higher N content might cause a reduction in seed oil content by declining the stem sugar level (Connor and Sadras, 1992). As illustrated (Fig. 4), a lower sucrose level slowed down lipid biosynthesis, which led to the declining in seed oil content (Khan *et al*., 2018) (Table 1).

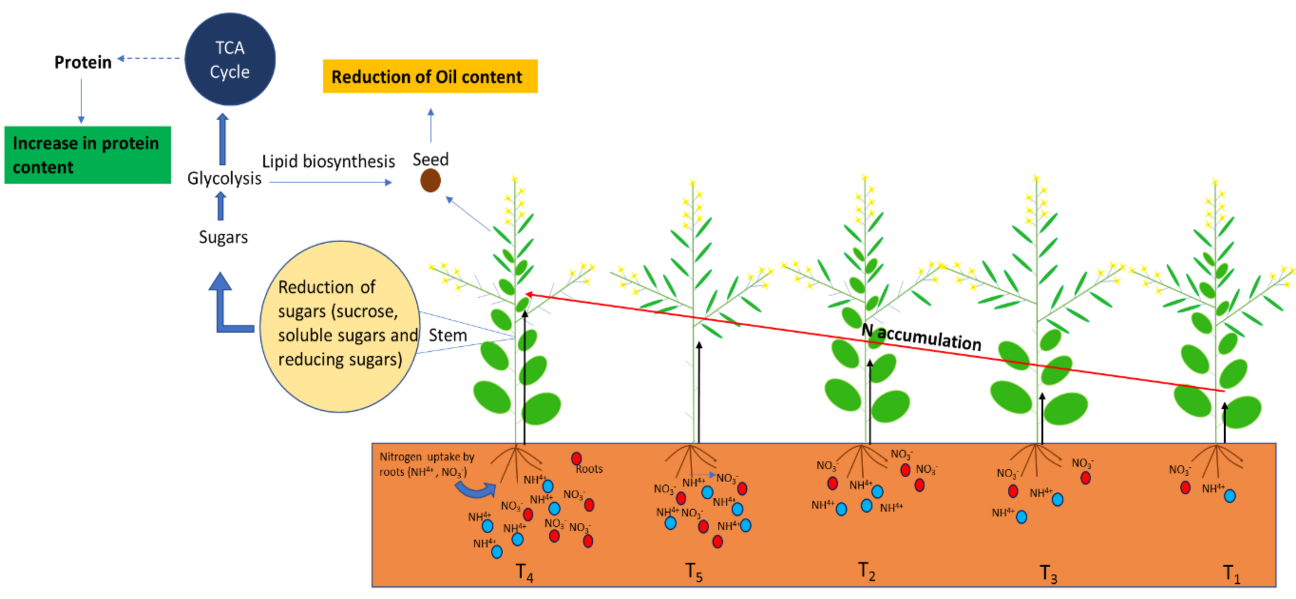

Fig. 4. Illustration of changes of nitrogen uptake and carbohydrates (sucrose and reducing sugar) accumulation in rapeseed stem, as influenced by defoliation and silique removal treatments. (T_1=control; T_2=50% branch silique removal; T_3=50% defoliation; T_4=100% branch silique removal; T_5=100% defoliation). The black arrow represents leaf and silique removal treatments on the nitrogen uptake in this paper. The figure clearly illustrates the significant improvement in silique removal treatment (T_4) compared to T_1. The blue arrows illustrate the carbohydrates accumulation effect on the oil and protein contents of the seed.

Defoliation and silique removal treatments (T_4 and T_5) increased seed protein concentration and decreased the seed oil compared to the control (T_1). These findings align with previous studies (McAlister & Krober, 1958; Openshaw *et al*., 1979; Schonbeck *et al*., 1986). The relationship between the oil and protein content was inversely proportional, which was confirmed by previous researcher's results in the sunflower; an increase in protein content led to a decrease in oil content (Andrianasolo *et al*., 2016). However, our results will be helpful to get deeper and better insights on the affective responses to leave cutting (defoliation) and silique cutting management on carbohydrates availability, seed protein, and oil concentration of rapeseed cultivars. However, there is a dire need to evaluate the impact of leaf-cutting (defoliation) and silique-cutting treatments on the molecular mechanisms that regulate carbohydrate availability and seed quality in rapeseed.

Conclusion

This study evaluated the effect of defoliation and silique removal on the seed yield and its quality. The defoliation and silique removal treatments during the seed filling stage of rapeseed increased the nitrogen accumulation from soil and also increased the remobilization from other parts of the plant toward the seed. Furthermore, high nitrogen accumulation and nitrogen remobilization declined the stem's sugar level, which is necessary for fatty acid biosynthesis in the seed. The study found that seed protein content could be increased by defoliation and silique removal. Moreover, there is need to explore the new ways by which seed oil content should also increase by focusing on nitrogen and carbon metabolism. Our next study will focus on this.

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