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Effects of siliqua position on physico-chemical composition of canola (*Brassica napus* L.) seed

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Abstract

The difference of time in opening of flowers and distance from ground may affect physicochemical characteristics of canola (*Brassica napus* L.) seed. A study pertaining to investigate the effect of siliqua position on physico-chemical composition of seed was conducted during 2008-9. The seeds of canola variety Bulbul-2000 were sown during month of October 2008. Before the start of flowering 300 plants were selected to make three replications of 100 plants (in three rows) in each replication. The siliquas on main stem of each plant were labelled after shedding of petals on every second day starting from lowest siliqua, which continued untill the last siliqua on upper most portions. In total 12 siliqua positions were labelled on the main stem. Labelled siliquas on each position were harvested separately. Seeds per siliqua, thousand seed weight, oil and protein content were determined from harvested siliquas, separately. Results revealed consistent reduction in number of seeds/Siliqua (23.38 to 14.33) , 1000 seed weight (3.88 to 2.92 g) and oil content as siliqua development progressed upward to the top (40.01 to 48.56 %), hence inferred an inverse relationship to the siliqua position. However, protein content depicted reverse trend to the above parameters and increased towards the top, thus exhibited a linear relationship to siliqua position.

Keywords: *Brassica napus*, oil, protein, siliqua position, photosynthates.

Introduction

Brassica napus L. (Canola) is a bright yellow flowering member of the family Brassicaceae. It contains 40-45 % oil content and 36-40 % protein in meal (Amin and Khalil, 2005). Growth and development of canola is continuous but can be divided into easily identifiable growth stages. Seed develop in a two celled, elongated capsule called siliqua with prominent mid-vein (Bailey, 1976). Approximately 15-25 seeds/siliqua are commonly produced. Seed development is first seen on the lowest position of the main stem. During flowering and siliqua set, the relation among source and sink regulates the convenience of assimilates essential for seed filling (Hua et al., 2012; Diepenbrock, 2000). Similarly C/N ratio in the seed which is useful physiological indicator for selecting higher seed oil content in the Canola could be considered for selecting best line (Hua et al., 2012). Usually photosynthates are transported from maternal to filial tissues, then they are delivered to the extra cellular space (via apoplast) separating the two generations, prior to uptake from the apoplast into the tissues of the embryo/endosperm (Patrick and Offler, 2001). The osmotic atmosphere of seed tissues has a strong effect on photosynthate transport into empty seed, a turgor hemostat mechanism may help to maintain high solute concentrations in the seed apoplast. The apoplast surroundings of seed tissues may also stimulate synthesis of storage proteins and be involved in the avoidance of precocious germination (Wolswinkel, 1992). Genotypic and ecological variables may affect the amount and excellence of canola oil (Flagella et al., 2002). Between the environmental parameters upsetting the concentration of canola oil, temperature is one of the most important ones, declining the seed oil content if it increases (Pritchard et al., 2000). The optimum daily temperature for canola flowering is 20 °C (Chen et al., 2005). In general seed expansion is divided into three stages: In stage 1, seed weight is small and starch and ethanol soluble compounds accounts for 80 % dry matter, in stage 2, seed enlargement takes place and storage oil and proteins deposits account for 40 % and 20 % dry matter correspondingly at the end of this phase. Starch, glucose and fructose are utilized in this process. Stage 3 is mainly concerned with the deposition of oil and protein in fixed magnitude. Sugars are transferred from hull toward seed to maintain this growth. In the seeds, the impartial lipid increases from 20 % of the total lipids in phase 1-93 % at maturity. The proportion of structural lipids declines as the storage lipids increases. (Norton and Harris, 1975). Seed development and enlargement takes place at different times, at different positions (distance from main stem, the photosynthates producing region) and at different temperatures (Bewley and Black, 1994). Thus, presumably

Table 1. Mean temperature and Total Precipitation during growing season.

Months	Mean Temperature (˚C)	Precipitation (mm)
October	23.1	23
November	16.6	15
December	13.1	71.5
January	11.4	59.3
February	13.4	49.2
March	17.5	58.6
April	21.7	116.2
May	27.6	35.6

Source: Meteorological Office, Rawalpindi.

difference of time, position and temperature affect physicochemical characteristics of the seed. At the initiation of flowering maximum leaf area in synthesizing ample quantity of photo assimilate which is transported to the sink. However, towards the end of flowering, the total leaf area decreases with yellowing of lower leaves while at the same time demand by sink increases which creates competition of assimilate partitioning. The information related to effect of siliqua position on physicochemical characteristics of seed are limited in primary literature sources. Therefore, present study was designed to investigate the effect of siliqua position on physico- chemical characters of *Brassica* seeds.

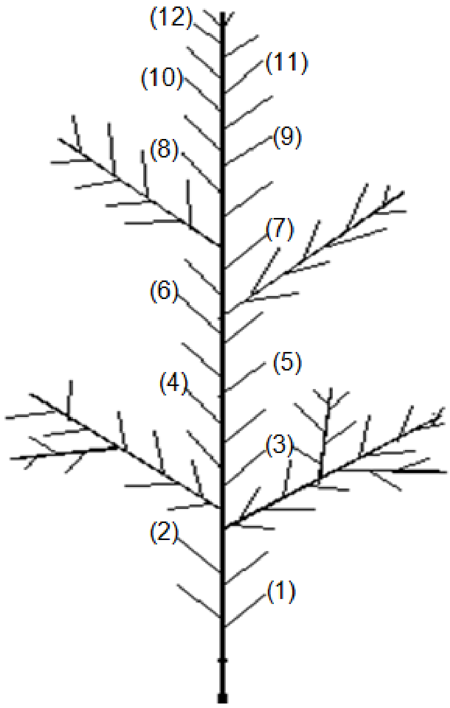

Fig 1. Schematic sampling locations of siliquas on main stem of canola plant

Results and Discussion

Seeds/siliqua

Siliqua position significantly ($p < 0.05$) affected seeds/siliqua (Table 2). The maximum (23.38) number of seeds/siliqua was observed at the lowest position that decreased progressively towards the top. The minimum (14.33) number of seeds per siliqua was recorded at top position. A difference of 74.97 % for seeds per siliqua was visible between lowest and the top most position. The maximum number of seeds per siliqua at lowest position indicated that conditions at the time of siliqua development at lower portion were more favorable i.e. temperature and maximum availability of photosynthate as most of the leaves were green having maximum photosynthetic capacity, thus resulted maximum seed setting. It has been concluded by Gupta et al. (2009) that the temperature affects relations between source and sink which regulates the availability of assimilates necessary for seed filling. Proper vegetative growth is necessary to supply photo assimilate to developing seeds (Gul and Ahmad, 2004; 2006). The progressive reduction in number of seeds/siliqua at upper positions may be the effect of temperature variation, the optimum daily temperature for flowering in canola is 20 ^{0}C (Chen et al., 2005). In our study, average daily temperature at first day of labeling siliqua was 15.75 °C which progressively increased to 19.75 °C at the 8th siliqua position. Thus, the difference of 4 °C in temperature may have caused possible reduction in seeds/siliqua. However, this may not be the only factor to cause this variation. Results of present study are similar to Gunasekare et al. (2006) who observed significant decrease in seed setting through increase of temperature in rapeseed.

1000 Seed weight

The 1000 seed weight (g) obtained from different siliqua position exhibited statistically significant differences ($p <$ 0.05) (Table 2). Seeds at the lowest siliqua position had the maximum (3.88 g) 1000 seed weight which decreased to the minimum (2.93 g) at the topmost siliqua, thus showed a reduction of 75.19 % in 1000 seed weight between upper most and lowest siliqua positions. These variations in seed weight may be due to competition for photo-assimilates at later stages of siliqua development and climatic factor like temperature and moisture availability. Higher temperature during reproductive stage significantly inhibits the import of photosynthates and thereby decreases the sink strength. Reduced availability of photosynthate and late maturation of seeds of upper siliquas as compared to lower siliquas may be the cause of decrease in 1000 seed weight. Similar observation was reported by Munshi and Kumari, (1994), in which they found healthier lower siliquas having higher seed weight than upper siliquas. Variability in seed weight throughout seed development in different whorls of sunflower head was recorded by Gupta et al. (2009). Similarly, Kaleem and Hassan, (2010) observed heavier seeds in outer circles of sunflower than in inner circle, while Tashiro et al. (1990), reported differences in seed weight among lower part capsules and upper branch capsules in sesame.

Table 2. Effect of Siliqua position on Seeds per pod and TSW of Brassica.

Siliqua position	Seeds/pod	TSW (g)	Oil content (%)	Protein content (%)
1	23.38A	3.88A	48.56A	38.18H
2	22.52B	3.77B	47.48B	39.26G
3	21.55C	3.66C	47.29B	40.42F
4	20.53.D	3.57CD	46.09C	41.19E
5	19.49E	3.49DE	45.93C	41.53E
6	18.58F	3.43EF	44.11E	41.95D
7	18.13F	3.38FG	42.41F	42.49C
8	17.53G	3.31GH	42.79F	42.88B
9	16.46H	3.24H	41.86G	43.23B
10	15.43I	3.12I	40.54H	43.72A
11	14.49J	3.04I	40.09H	43.82G
12	14.33J	2.92J	40.01H	43.95G

(Different letters in the same column show a significant difference at P < 0.05).

Thus irrespective of crop, phenomena of assimilate transportation seems to be the same *i.e.*, longer the distance from source lesser will be accumulation in sink. Inverse relationship (Fig. 2) between siliqua position, seeds per siliqua and TSW is supportive to above assumption.

Oil content

Oil content in seeds obtained from siliquas at lowest position was significantly higher than seeds from rest of siliqua positions (Table 3). Oil content from seeds of lowest siliqua position was maximum (48.56 %), which decreased consistently to the top most siliquas, giving the minimum (40.01 %) oil content.. In present study, the siliquas emerging at relatively low temperature (15.75 °C) accumulated higher oil content, however, at later stages near crop maturity average daily temperature increased from 15.75-19.75 °C, which may have caused reduction of oil content. The siliquas emerged and developed earlier remained for longer period of time as sink thus having shared of assimilates from the source for longer duration, consequently accumulated higher oil content. In present study oil accumulation exhibited inverse relationship to siliqua position (Fig. 3). A comparable pattern of variability among various positions on the main stem in mustard seed was observed by Munshi and Kochar (1994) and Munshi and Kumari (1994), with the conclusion that oil proportion in the pods decreased with the phase of siliqua growth, likewise, in soybean central capsules had seeds with less oil content than seeds from lateral capsules (Gularia et al., 2008). In addition, Gupta et al. (2009) observed higher oil yield in peripheral whorls than central whorls in sunflower.

Protein content

In present study, statistical differences were recorded among different siliqua positions for protein content ($p < 0.05$) (Table 3). The maximum (43.72 %) protein content was observed at the top siliqua while minimum (38.18 %) obtained from the lowest. The siliquas developed early in the season accumulated lesser protein as compared to those developed at later stages. Protein accumulation is considered having inverse relationship with oil content. The inverse relationship (Figure 3) between siliqua and protein content is supportive to above assumption. Our findings are in line with earlier findings of Pritchard et al. (2000), they observed higher protein content in upper siliquas than lower siliquas due to high temperature.

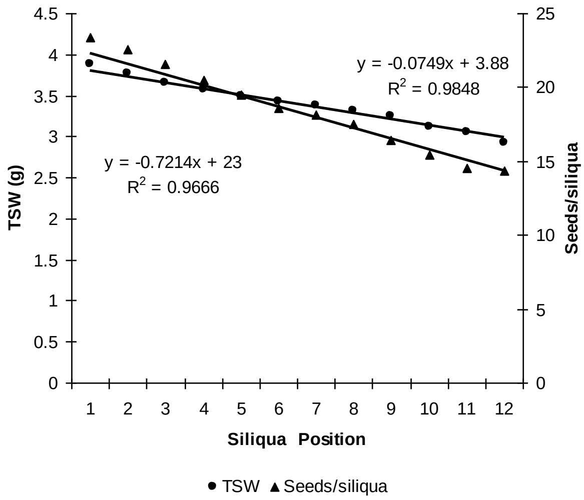

Fig 2. Relationship between siliqua position, seeds per siliqua and TSW of canola

Likewise, these results are in conformity with the findings of Chung et al. (1995) reporting sudden increase in protein content after 12 days of flowering. It can be concluded from present study that the seeds developed in siliqua at lower position having lesser distance from ground and source were more productive in terms of various physicochemical characteristics in comparison with those developed at upper siliqua positions. Therefore, we may conclude that it is important to develop cultivars having some definite flowering pattern with maximum flowers at lower positions and lesser distance from the source.

Materials and methods

Influence of siliqua position on physico-chemical characteristics of *Brassica* seed was studied through field experiment conducted at Pir Mehr Ali Shah, Arid Agriculture University, Rawalpindi, Pakistan during 2008-9. The site was disked and ploughed twice before planting. Recommended doses (100:50:50 kg ha^{-1}) of nitrogen, phosphorus and potassium were applied at the time of last ploughing. Seeds of *Brassica napus* L. variety Bulbul-2000 were used as test material. Seeds were sown with the help of hand drill in plot size of 6 × 3 meters on October 16, 2008. The distance between rows were maintained at 20 cm and between plants at 5-6 cm after thinning at 3-4 leaves stage. Before start of flowering 300 plants were randomly selected with 100 plants in each replication (in three different rows). On the

Table 3. Effect of Siliqua position on Oil and Protein contents of Brassica.

Siliqua position	Oil content (%)	Protein content (%)
1	48.56A	38.18H
2	47.48B	39.26G
3	47.29B	40.42F
4	46.09C	41.19E
5	45.93C	41.53E
6	44.11E	41.95D
7	42.41F	42.49C
8	42.79F	42.88B
9	41.86G	43.23B
10	40.54H	43.72A
11	40.09H	43.82G
12	40.01H	43.95G

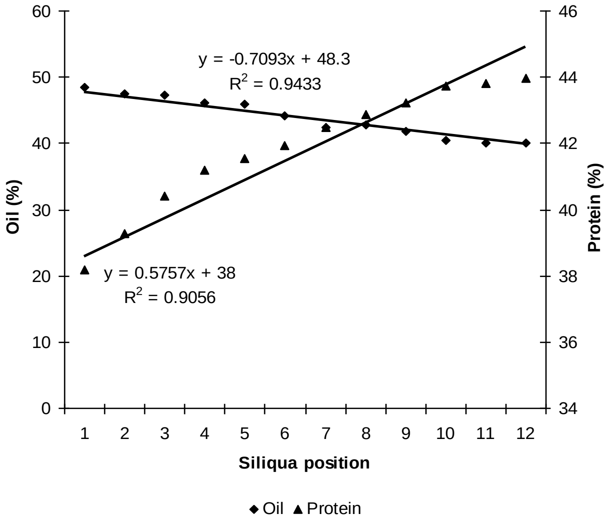

Fig 3. Relationship between siliqua position, oil and protein content of canola.

appearance of siliqua on main stem labelling was done on the day of emergence, starting from lowest point. The first labelling was called as first treatment, which was done on February 22, 2009. Similarly, twelve treatments were recorded on twelve different dates on the basis of emergence of siliquas. Approximately 100 siliquas were labelled on each date. Labelling continued till the end of flowering on main siliqua bearing raceme. The siliqua appearance ended on March 27, 2009 (Fig. 1). Thus experiment had 12 treatments and three replications arranged in completely randomized design. At maturity, all the labelled siliquas with respective dates were removed and kept separately. These labelled siliquas were thrashed manually and total number of seeds/ siliqua were counted and weighed separately. Seed from each position were separately analasyed for oil and protein. Oil content was estimated on Soxhelet apparatus following AOAC (Association of Official Analytical Chemists) protocol (1990) and protein content was measured following Bradford assay (Bradford, 1976). Data were statistically analysed using analysis of variance technique with the help of M-Stat-C version 2.1 (Freed and Eisensmith, 1986). Duncan's multiple range test was applied to compare the means at $P = 0.05$ **(**Montgomery**,** 2001).Weather data of crop duration was obtained from Regional Meteorological Centre located near the experimental site (Table 1).

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