Biomass partitioning in canola (*Brassica napus*) and the characterization of leaf anatomical structures

by

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Abstract

Canola is the predominant crop grown on the Canadian Prairies, contributing about $26.7 billion to the economic activities per year in Canada. In 2023, Canada produced approximately 18.3 million tonnes of canola; however, the Canola Council of Canada set a goal of producing 26 million tonnes by the year 2025. One approach to achieve this is the manipulation of plant physiological properties. Assimilates produced through photosynthesis contribute significantly to the growth and development of canola plants by effectively partitioning resources into sink organs. Enhanced resource partitioning represents a novel approach for the improvement of canola for high yield under field conditions. This MSc thesis research was undertaken to investigate i) biomass and resource partitioning, and ii) morphological characteristics of leaf anatomical traits of a diverse set of canola accessions from the Canola Breeding Program at the University of Alberta. The study was conducted across five site years i) West-240 (2021), ii) St. Albert (2022), iii) West-240 (2023), iv) St. Albert (2023), v) CDC-North (2023), and evaluated 168 canola accessions from seven different pedigree groups and two checks (L255PC, 45H33). The major parameters investigated for this study included plant biomass (total biomass, leaf, stem, root, root-shoot ratio), leaf area index, thousand seed weight, seed yield, and anatomical parameters of the adaxial and abaxial leaf surfaces (stomatal density, epidermal cell density, trichome density, and stomatal index), under different climatic conditions. Results demonstrated that environmental factors exerted significant effect on biomass allocation and yield parameters. Experimental sites had significant effect on all biomass and resource partitioning variables with canola accessions performed differently at different sites. In summary, at CDC-North site, all canola accessions including the checks produced significantly greater biomass (total biomass, leaf dry weight, stem dry weight) i.e. source and higher sink weight (yield, thousand seed weight). Canola at this site also showed significantly lower root/shoot ratio indicating efficient resource allocation towards the above ground biomass and yield. Several canola accessions outperformed the checks demonstrating superior source activity and efficient resource partitioning; however, same accessions also performed differently at different sites. The West-240 site experienced drought condition in 2021, which impacted the seed yield; therefore, yield at this site was significantly lower than at other sites. Biomass was also lower and root/shoot ratio was higher in this site. Among the anatomical traits, the stomata, which is crucial for gas exchange and water regulation, exhibited significant variation among the different canola pedigree groups. Correlation and regression analyses elucidated strong positive relationships between stomatal density and epidermal cell density, implying that they may have co-evolved. Overall, this study provided valuable insights into the physiological and morphological aspects of the canola leaves, and the knowledge gained from this study can facilitate crop management strategies and help breeding efforts for the development of resilient and productive canola cultivars.

Preface

This thesis represents an original contribution by me, Saima Jahan Liza, towards the attainment of the degree of Master of Science in Plant Science. The meticulous planning of all research endeavors was spearheaded by my esteemed supervisor and co-supervisor, Dr. Linda Gorim and Dr. Habibur Rahman. Their invaluable comments and suggestions throughout the thesis writing process have been indispensable.

I attest that no part of this thesis has been previously published, save for conference abstracts corresponding to presentations delivered during my M.Sc. program.

The experimental design outlined in Chapter 2 was conceptualized by Salvador Lopez, with comprehensive field trial planning and preparation facilitated under the expert guidance of Dr. Linda Gorim. My involvement in seed preparation and seeding, alongside Salvador Lopez and Karanjot Gill, was instrumental in laying the groundwork for data collection. I extend my heartfelt appreciation to my lab mates and summer students for their efforts in data collection, with special mention to Fernando Guerrero Zurita, Sumedha Vaishnavi Nallantighal, Priscillar Wenyika, Jedida Chirchir, and Molla Hailu for their contributions to sample collection.

Data analysis was diligently undertaken by me, with the invaluable assistance of Dr. Rebecca Oiza Enesi, Dr. Berisso Kebede, and Fernando Guerrero Zurita, who provided invaluable guidance throughout the analytical process. Special acknowledgment is extended to the research staff at all sites, whose unwavering support was instrumental in the successful execution of the study.

Chapter 3 involved meticulous slide preparation by Dr. Linda Gorim and Karanjot Gill at the West-240 site (2021), with subsequent data collection and analysis undertaken by me. I am deeply grateful to Heather Vandertol-Vanier and Dr. Urmila Basu for their invaluable training in the use of the microscopy room.

Financial support for this research was generously provided by Results Driven Agriculture Research (RDAR), the Alberta Canola Producers Commission, and Western Grain Research Foundation (WGRF). The canola accessions utilized in this study were developed by the Canola program of the University of Alberta under the supervision of Dr. Habibur Rahman.

Dedication

To my beloved parents,

Your endless love and support have been my guiding light. This thesis is a tribute to your unwavering belief in me.

And to my dear husband,

Your encouragement and love have fueled my journey. This thesis reflects our shared dreams and commitment.

With heartfelt gratitude,

Saima Jahan Liza

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List of abbreviations

%	Percent
(W × S) × R	(Winter canola x Spring canola) x Rutabaga
°C	Degrees celcius
45H33	Roundup ready canola
AAC	Agriculture and Agri-Food Canada
ANOVA	Analysis of Variance
approx.	Approximately
ATP	Adenosine Triphosphate
BBCH	Biologische Bundesanstalt, Bundessortenamt und Chemical Industry
bu/ac	Bushels per acre
C	Carbon
C_3	C_3 plant
C_4	C_4 plant
CDC-N	Crop Develeopment Centre- North
chl	Chlorophyll
cm	Centimeter
CO_2	Carbon dioxide
DTF	Days to Flowering
DTM	Days to Maturity

e.g.	For Example,
EDAB	Abaxial epidermal cell density
EDAD	Adaxial epidermal cell density
Fv/Fm	The maximum quantum efficiency of photosystem II
GAR	Gross assimilation rate
HI	Harvest index
kg/ha	Kilogram per hectare
kPa	Kilopascal
L255PC	LibertyLink canola
LAI	Leaf area index
lb/acre	Pound per acre
LDwt	Leaf biomass
LEF	Linear electron flow
LSD	Least significant difference
m	Meter
m2	Meter square
mm	Millimetre
N	Nitrogen
NASA	National Aeronautics and Space Administration
NPQ	Non photochemical quenching
NPQfast	Rapidly reversible photoprotection
NPQslow	Photoinhibition of photosystem II

ns	Nonsignificant
P	Phosphorus
LMA	Leaf mass area
PC	Principle component
PCA	Principle component analysis
PE	Photosynthetic efficiency
PSII	Photosystem II
QTL	Quantitative trait loci
R/S	Root/ Shoot ratio
RDwt	Root biomass
Rubisco	Ribulose 5-bisphosphate carboxylase/oxygenase
RuBP	Ribulose 1,5-bisphosphate
S × Oleracea	Spring canola x *Brassica oleracea*
S × R	Spring canola x Rutabaga
S × Rapa	Spring canola x *Brassica rapa*
S × S	Spring canola x Spring canola
SDAB	Abaxial stomatal density
SDAD	Adaxial stomatal density
SDwt	Stem biomass
SIAB	Abaxial stomatal index
SIAD	Adaxial stomatal index
SLA	Specific leaf area

SUMP	Suzuki’s Universal Micro-Printing
TDAB	Abaxial trichome density
TDAD	Adaxial trichome density
TDwt	Total biomass dry weight
TKW	Thousand kernel weight
USA	United States of America
UV	Ultraviolet
VPD	Vapor pressure deficit
W × S	Winter canola x Spring canola
WUE	Water use efficiency

Chapter 1 Literature Review

1.1 Introduction

Canola (*Brassica napus*), also known as rapeseed, plays an important role in producing edible vegetable oils and is favoured for its low saturated fat content (Canola Council of Canada, 2023). It is a variety of rapeseed that was developed in the 1970s by Canadian plant breeders; the name "canola" is a contraction of "Canadian oil, low acid" (Canola Council of Canada 2022). Introducing canola oil in diets has shown to have positive impact on human health by reducing blood cholesterol levels, improving insulin sensitivity, and increasing tocopherol level (Lin et al., 2013). Therefore, canola oil has gain popularity over the years and it has become the second largest oilseed crop (Raymer, 2002). Canola seed oil contains less than 2% erucic acid and seed meal contain less than 30 micromoles of total glucosinolates per g of meal (Kris-Etherton et al., 2000). In Canada, the average canola yield has consistently increased in recent years, accounting for about 25 % of total farm receipts. By 2025, the industry aims to increase yields to 52 bushels/acre for a total production of 26 million tonnes to meet the increasing global demand for canola oil (Canola Council of Canada, 2014). Intensive breeding to increase canola yields started in the 1990's (Kirkegaard et al., 2021). This oilseed crop has been improved by using different modern technologies including genetic engineering, focused primarily on seed yield, stress tolerance, and herbicide resistance (Ton et al., 2020). The production of harvestable crops per unit area that was harvested is known as yield which is very important determinant for the performance of a crop under field conditions (Fischer, 2015). Yield comes from the harvestable parts of the plants as the photosynthetic assimilates are partitioned into different organs including seeds (Marcelis et al., 1998). For the growth and development of a plant, photosynthesis is the most important process by which plants converts light energy into chemical energy (Evans, 2013). Carbon assimilates are produced via photosynthesis in the source organ of the plants and the main factors associated in this process are carbon dioxide, light, temperature, nutrients, and water (Fatichi et al., 2014). Photosynthesis process in crop plants positively correlates with plant biomass, which becomes evident from the enhanced vegetative growth and distribution of the photosynthetic products to the sink organs to initiate the growth of harvestable organs (Peng et al., 1991). Since photosynthesis and the partitions of photosynthetic assimilates are directly related to plant growth and development it has become important to further investigate these parameters for crop yield improvement. Genetic approaches to crop improvements are at saturation in many crops (Wu et al., 2019); however, future research should focus on efficient resource capture, utilization, and conversion by crops (Murchie et al., 2008). Research on photosynthesis in forest species has been conducted (Larcher et al., 2016); however, this is not common in crop plants. Therefore, it is important to focus on enhancing photosynthetic efficiency and growth rates in plants. However, Long et al. (2006) raised the question of whether photosynthesis can improve the yield of a crop. To address this, a review conducted on the progress in seed yield over the years in wheat, rice and maize demonstrates a positive effect of enhanced photosynthesis on yield (Fischer & Edmeades, 2010). Several research studies have been conducted on photosynthetic improvement of the crop plants, and this includes i) genetic engineering of photosynthesis through manipulation of phytocyanin proteins (Long et al., 2015; Zhang et al., 2017); ii) improvement in Rubisco (ribulose-1,5-bisphosphate (RuBP) carboxylase/oxygenase) enzyme (Whitney et al., 2011; Parry et al., 2013); iii) maximizing photosynthetic efficiency by improving antenna size (Ort et al., 2011) vi) improvement of C_3 photosynthesis (von Caemmerer & Evans, 2010); and v) creating disease resistant crop by manipulating photosynthesis for better crop performance (Yang & Luo, 2021). Photosynthesis takes place at source organs and the sink organs accumulate photosynthetic products, making source-sink relationship an important aspect of crop productivity. The significance of source-sink interaction and resource allocation in governing plant growth is crucial for the identification of balance between source and sink organs to unlock the potential yield gains (White et al., 2016). Limitations in source or sink activities result in poor performance of the crops (Zhang & Flottmann, 2018). A study with modern wheat demonstrated that the breeding efforts have reduced the strength of sink, resulting in increased grain weight and seed yield (Acreche & Slafer, 2009). Research on source-sink manipulation for increasing crop yield is on the rise and researchers are making substantial progress in different crops including canola, rice, wheat, sugar beets, chickpea, and grasses (Peterhansel & Offermann, 2012; Slewinski, 2012; Smith et al., 2018). Therefore, the evaluation of genetically diverse canola germplasm for source-sink relationship via biomass allocation and resource partitioning is imperative.

Source organs regulate gaseous compounds of photosynthesis through stomatal conductance (Miner et al., 2017). Stomatal conductance, which is the opening and closing of stomata, regulates gas exchange between the plant and environment, affecting both photosynthesis and transpiration rates, and thus, influence the overall balance between source and sink activities within plants (Gago et al., 2016). Stomata are the small openings or pores on the surface of plant leaves, stems, or other organs that are surrounded by guard cells. These guard cells play a vital role in physiological processes including gas exchange, photosynthesis, and transpiration. Given the importance of stomata in physiological and photosynthetic processes, it is imperative to gain more understanding of how they correlate with different anatomical structures including epidermal cells, and stomatal apparatus (Franks, & Farquhar, 2007). Plants carry a defense mechanism against herbivores through their source and sink organs including trichomes, thick leaves, thorns, and toxic chemicals, and they also indirectly enhance their protection by releasing volatiles that attract natural enemies of pests (War et al., 2012). Trichomes are small hair-like structures found on the surface of various plant parts including leaves, stems, and sometimes flowers. Trichomes are of particular interest in canola plants due to their role in various physiological and ecological functions. Trichomes can protect plants from heat and ultraviolet (UV) rays, facilitate dispersal, and play a dual role in herbivores' defense (Hanley et al., 2007). By regulating the stomatal process, trichomes can sustain photosynthetic performance and reduce the likelihood of oxidative stress (Paulino et al., 2020). A higher ratio of trichomes to stomata contributes to improved water use efficiency (Galdon-Armero et al., 2018). Different kinds of trichomes, such as glandular and non-glandular, act differently for insects and herbivores; glandular trichomes can release toxins and non-glandular can prevent insects' contact with plants (Fürstenberg-Hägg et al., 2013). Species with higher glandular trichome density exhibited reduced sensitivity to ozone stress with less visible ozone damage, highlighting the importance of glandular trichomes as a chemical barrier against ozone toxicity (Li et al., 2018). Therefore, an understanding of the presence or absence of different anatomical structures (stomata, trichomes, and epidermal cells) in canola germplasm is crucial for further improvement of this crop.

This project aims to investigate the relationship between biomass and resource partitioning abilities of the source and sink organs as well as anatomical characterisation (stomata, epidermal cells, trichomes) in different canola accessions, as these factors play crucial roles in plant physiology and productivity. By characterizing these traits, we can identify canola accessions with superior resource utilization and potentially improve crop yield through targeted breeding efforts.

1.2 Importance of canola as an oilseed

Canola (*Brassica napus* L. cv. ‘Canola’) belongs to the rapeseed group and the global production of rapeseed by 2022/2023 was 88.85 million metric tonnes, which was the second-largest oil-producing crop (USDA, 2024). Canola is a globally important crop and its collective significance lies in its nutritional benefits, economic contributions, agricultural advantages, environmental impact, and role in enhancing global food security (Downey & Bell, 1990; Friedt et al., 2018; Rempel et al., 2014; Shahidi, 1990). In Canadian agronomy, canola is one of the most important oilseed crops. Approximately 43000 crop producers grow canola in Canada on almost 20 million acres of farmland and produce about 20 million tons of canola seeds every year; the Provinces of Alberta, Saskatchewan, and Manitoba are the main producers of this oilseed crop (Canola Council of Canada, 2022). As an oilseed crop, canola has a great impact on the Canadian economy contributing about 29.9 billion dollars per year (Canola Council of Canada, 2022). Canola is a relatively new crop in Canada. The majority of the canola cultivars grown belongs to *Brassica napus* in the family, Brassicaceae (Argentine canola); a small amount of *B. rapa* (Polish canola) and condiment mustard *B. juncea* are also grown (Canola Council of Canada, 2022). Currently, more than 100 canola cultivars are registered in Canada (Canola Council of Canada, 2022); most of them carry LibertyLink, Roundup Ready, TruFlex or Clearfield herbicide tolerance traits (Alberta Seed Guide, 2022). LibertyLink canola can provide control of over 35 broadleaf and grassy weed species (including volunteer crops), and produce high yield and carry disease resistance; Roundup Ready canola cultivars were developed to provide resistance to glyphosate herbicide; cultivars show good standability, disease resistance, and yield potential; TruFlex canola was developed upon Roundup Ready Canola to provide maximum weed control and higher seed yield; growing of Clearfield canola can provide control of annual grassy and broadleaf weeds (Canola Council of Canada, 2022). Based on flowering behavior, *B. napus* can be grouped into three categories: spring, semi-winter, and winter. A study carried out in Sweden from 1976 to 1979 on different *Brassica* oilseed crops showed that significant difference exists between the species and growth habit types for seed yield. The winter *B. napus* yield (48 bu/ac) was greater than winter turnip rapeseed (*B. rapa*) (36 bu/ac), and spring *B. napus* yield was (32 bu/ac) greater than the spring turnip rapeseed (*B. rapa*) (25 bu/ac) (Canola Council of Canada, 2022). Therefore, wherever possible, it is important to grow the higher-yielding type to meet the global demand of canola oil (Wang et al., 2020).

1.3 Source-sink relationship

In 1928, Mason and Maskell proposed the theory of plant source-sink relationship where the source is described as the producer that exports resources, and sinks, as the importers of the plant's resources. In seed crops, the contribution of the sink (carbohydrate) and the assembling of the carbohydrate by the grains (sink) determines crop yield (Zhang & Flottmann, 2018). The source and sink organs of a plant are completely dependent on each other. Source and sink organs are allied with biomass partitioning. Carbon fixation in plant cells is linked to the plant's source-sink mechanism, which helps in determining the growth and development of plant biomass. The same organ of a plant can be both source and sink at the same time on a situational basis, such as the mature plant leaves can be the source of carbon but sink for nitrogen (White et al., 2016). Allocation of resources into source and sink organs are the underlining mechanisms in biomass partitioning and allocation into leaf, stems, and roots. Mature leaves which contain chlorophyll are the major source of carbon, while phloem parenchymatous cells of leaf and other organs act as carbon and nitrogen reserve pool. Roots or tubers are the sink or consumers of carbon while tubers, fruits or seeds act as a sink of organic and inorganic nitrogen (Chang et al., 2017). The function and stability of sources and sinks are highly dependent on the activities of assimilates (Ho, 1988). In canola, seed weight and yield decrease when assimilates are not readily available during seed-filling stage (Zhang & Flottmann, 2018). A review by Smith et al. (2018) demonstrates that the yield and nutritional quality of a crop is highly influenced by the source-sink relationship. The xylem and phloem, forming the plant's vascular transport system, connect source and sink organs, facilitating the flow of biochemical components across tissues (Chang et al., 2017). In canola, silique is very important for seed yield; they are chlorophyll-bearing organs and are photosynthetically active. Siliques are typically long and narrow and contain multiple seeds that are protected and nourished by the silique's walls until they are ready to germinate. More than 3000 species of the family Brassicaceae produce non-fleshy fruits and many of them are siliques which become the major sink organ that stores several important assimilates like carbon and nitrogen (Bennett et al., 2011). Li et al. (2019) investigated the effect of maternal genotype on seed weight in *B. napus* and demonstrated that the silique wall is the major place of photosynthesis contributing to seed weight, the most important trait for seed yield in this oilseed crops.

1.3.1 Resource partitioning and source-sink relationships

Resource partitioning refers to the allocation of assimilates and other resources among different plant organs based on their respective demands and priorities (Fernie et al., 2020). In spring canola, considerable variation exists in cultivars for resource partitioning; in this, a greater allocation of photoassimilates into seeds results in increased yield (Arvin et al., 2014). Oilseed rape or canola plants rely heavily on translocation of pre-anthesis assimilates to siliques highlighting the importance of resource partitioning for silique and seed development (Papantoniou et al., 2013). Seasonal variation and weather conditions can affect the source-sink relationships and the translocation of assimilates (Papantoniou et al., 2013). Elevated soil salinity significantly diminishes canola growth and yield by impeding carbon and nitrogen accumulation, which results from an altered resource partitioning (Zuo et al., 2019). A study conducted by Yesari et al. (2009) with several soybean cultivars under sink-limited (removal of siliques) conditions showed yield difference due to this condition. This indicates that photosynthetic assimilates might have been reallocated from sources to other parts of the plant, such as vegetative organs, due to the altered source-sink dynamics. This research group also reported that upon removal of flowers of the soybean plants, yield losses were compensated by increasing seed weight and adjusting resource allocation towards yields though source-sink interaction. Zhang et al. (2020) developed a model to predict the aboveground biomass partitioning in rapeseed. They found a significant correlation between biomass partitioning coefficients and physiological development; this highlighted the relationship between resource partitioning and source-sink dynamics. In a review, Chang et al. (2017) proposed a bottom-up and top-down approach (Integrative systems approach: a new model proposed to link molecular mechanisms directly to physiological source-sink interactions by treating plant growth and development as a single problem. Universal application and research tool: this aims to enhance both basic and applied research by characterizing molecular events and key processes influencing source-sink dynamics, ultimately aiding in crop yield enhancement) to study source-sink relationships in crop plants to improve agronomic yields. Their models emphasized the need of developing a framework that includes factors like carbohydrate accumulation-induced inhibition of photosynthesis, N remobilization-induced leaf senescence, and C- and N-based sink development and maintenance.

1.3.2 Source-sink relationship and N mobilization

Nitrogen (N) is crucial for plant growth as it is a key component of chlorophyll, amino acids, and proteins, directly influencing photosynthesis, nutrient uptake, and biomass production. It is an essential macronutrient for plant growth and plays a critical role in source-sink relationships. Adequate nitrogen supply to the leaves can increase the rate of photosynthesis and the production of photosynthates, leading to the development of more sinks such as fruits and seeds; proper nitrogen mobilization is crucial for maintaining the source-sink relationship in crops and ensuring optimal plant growth and productivity (Evans and Clarke 2019). In *Brassica napus*, biomass accumulation to source-sink organs (leaf, root, stem, whole plant), photosynthetic nitrogen use efficiency, stomatal conductance, photosynthetic rate, water use efficiency (WUE) decreases when the availability of N and phosphorus (P) decreases due to drought conditions (Biswas et al., 2019).

1.3.3 Source-sink and relationship with yield

The source-sink relationship plays a pivotal role in determining overall yield, as source organs are responsible for photosynthesis and the production of assimilates, while sink organs utilize these assimilates for growth and development. This relationship has been studied for decades with many initiatives and research aimed at manipulating the source-sink relationship to increase crop yield, this received even a greater attention due to climate change and environmental issues (Chang et al., 2017). According to Zhang and Flottmann (2018), seed yield of spring canola in Australia show a positive relationship with the availability of source during seed-filling stage. Higher plant biomass including biomass at different phenological stages results in a greater number of silique and higher yield in western Australian canola cultivars (Zhang and Flottmann, 2016). The improvement of source-sink relationship is reported to result in higher yields and resource use efficiency in rice (Zhang et al (2021). Apart from genetic improvement, the improvement of source-sink relationship can be achieved through better cultivation and crop management practices. Studies in Southern Australia revealed that canola yield is mainly bound to source rather than sink during seed-filling stage (Zhang & Flottmann, 2015). Aye et al. (2020), and Lubis et al. (2003) reported that the yield potential of rice is more related to the source activity than the sink activity. According to Li et al. (2015), the size of tomato fruit is dependent on the source at fruiting stage. A study conducted in soybean in Iran indicated that source limitation (leaves elimination) causes reduced yields (Yasari et al., 2009). The transformation of assimilates to biomass, their distribution to grains, and regulation by sink organs are the key factors in determining crop productivity (Jeng et al., 2006; Ruan et al., 2010). In canola cultivars, variations in biomass and its partitioning are dependent on growth stages. At the vegetative stage, a greater percentage of biomass is distributed to the leaves rather than to the stem, while at the flowering stage, the reverse allocation occurs (Zhang & Flottmann, 2016). Generally, a deficient or insufficient sink capacity (i.e., small sink) relative to the size of the source (bigger source) can reduce crop yield by limiting the proper utilization of source components.

1.4 The process of photosynthesis

Photosynthesis is the most important physiological mechanism occurring in plants where light energy is converted into chemical energy (Stirbet et al., 2020). Plants' growth and metabolism are boosted through photosynthesis, facilitated via various reactions (Podgórska et al., 2020). Photosynthesis process is important for plant growth and development and occurs through CO_2 reduction and absorption of light by photosystem (Anelise et al., 2012). The light energy that is transmitted, transformed, and absorbed during photosynthesis is highly dependent on the cellular chlorophyll content (Cheng & Liu, 2010). Photosynthesis process takes place in chloroplast where a structure called thylakoid contains the chlorophyll pigment (Concepts of Biology, 2013). Photosynthesis reaction comprised of two cycles, which includes the light dependent cycle, that occurs in thylakoid; where light energy is converted to chemical energy, and a light-independent cycle or Calvin cycle, which occurs in the stroma and uses the chemical energy from the light-dependent cycle to produce sugar molecules (Concepts of Biology, 2013). In linear photosynthetic electron transfer chain, the integrated activity of photosystem I (PSI) and photosystem II (PSII) are the basis of the light reaction activity of photosynthesis (Podgórska et al., 2020). Although, the yield of a crop depends on several factors such as the environmental conditions, plant growth, and development dynamics, leaf photosynthesis plays a vital role and closely linked with crop yields (Wu et al., 2019). To develop a photosynthetically efficient high-yielding crop, several parameters related to enhanced photosynthetic activity need to be considered to select the most efficient crop cultivar. These parameters can be the amount of CO_2 absorbed by leaf and light-energy efficiencies, higher rate of carboxylation of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), mesophyll conductance for CO_2, and magnitude of electron transport (Wu et al. 2019). In the photosynthesis process, plants produce carbohydrates (starch and sucrose) by fixing photosynthetic carbon (Concepts of Biology, 2013). Stomata are present in mesophyll cells of plant leaf, which facilitate gas exchange and aids in the photosynthesis process (Nilson & Assmann, 2007; Xu & Zhou, 2008). The physiological mechanism by which chlorophyll enhances crop productivity and yield involves Rubisco in the stomatal carbon fixation process and this reaction, which requires ATP, is facilitated by chlorophyll pigments (Iqbal et al., 2014). Plant yield and several other physiological traits are influenced by chlorophyll content (Miglani et al., 2021). Therefore, to develop a higher-yielding canola cultivar, understanding photosynthetic efficiency is important, and this can be achieved through the measurement light use efficiency (Wang et al., 2020).

1.4.1 Photosynthesis in Siliques

Effective photosynthesis in plant siliques from the Brassicaceae family contributes significantly to assimilate production and plant growth, resulting in improved yield (Bennett et al., 2011). The silique wall of Brassica is one of the major places of photosynthesis, which play an important role in seed weight - the most important trait contributing to crop yield (Li et al., 2019). Silique also exert influence on seed composition (Bennett et al., 2011). In transgenic plants of the Brassicaceae family, the ratio of chlorophyll a/b and total chlorophyll content are inversely related, and the optimum chlorophyll a/b ratio show higher photosynthetic efficiency (Friedland, 2019). Higher yield associated with greater photosynthesis efficiency is not solely based on the change in chlorophyll a/b ratio, but influenced by other factors such as pronounced branching, optimal flowering, and high silique numbers (Friedland, 2019). A lower production of assimilates at flowering stage can result in fewer number of silique and, thus, can negatively affect canola yield even when seed weight is increased (Zhang, & Flottmann, 2018). Silique can also act as a sink that recycles protein, lipid, and starch during seed development (Bennett et al., 2011). However, canola yield can be limited by sink organs, especially when the flowering stage and silique development stage are affected (Zhang & Flottmann, 2018), findings related to the studies from other crops (Fischer, 1985). Zhang et al. (2016) reported that photosynthesis in alfalfa pods has a contribution to seed development while fulfilling assimilate requirements.

1.4.2 Photosynthetic traits

Photosynthetic traits are the features of plants that controls and impact their ability to carry out photosynthesis. These traits include chlorophyll content, carbon fixing mechanism, photochemical reaction center, electron transport chain, leaf anatomy, and stomatal conductance (Arntz & Delph, 2001). An analysis of quantitative trait loci (QTL) for photosynthetic traits, including maximal quantum efficiency (Fv/Fm), rapidly reversible photoprotection (NPQfast), and photoinhibition of PSII (NPQslow), using high-throughput chlorophyll measurements in *Arabidopsis thaliana* revealed variations in response to cold treatment (Oakley et al., 2018). In photosynthetic light reaction, electron flows through chloroplast, termed, the linear electron flow (LEF). LEF has great influence in photoinhibition of photosystem II (PSII) that is the maximum quantum yield of photosystem II (Fv/Fm) in shade-loving plant *Panax notoginseng* (Huang et al., 2018). Research have shown that the narrower antenna (light harvesting complex) size causes alteration in the PSII photochemical efficiency (Fv/Fm) creating photodamage, which can reduce the photosynthetic efficiency (Friedland, 2019). Non-photochemical quenching (NPQ) is essential for analyzing photosynthetic efficiency, as it dissipates excess light energy to prevent photodamage, with higher NPQ levels often indicating slower plant development during stress conditions (Friedland, 2019). NPQ and LEF declines in mint plants due to photodamage when exposed to increased light as they are highly sensitive to light (Kanazawa et al., 2021).

1.4.3 Photosynthesis metabolism under various stress

Plants' internal mechanisms including photosynthesis, carbon assimilation, biomass partitioning, and yield can react positively or negatively under different stresses such as heat, drought, heavy metal pollution, and carbon dioxide elevation (Gan et al., 2014; Elferjani and Soolanayakanahally, 2018; Wang et al., 2021). Being sessile, plants can undergo several mechanisms to cope with both biotic and abiotic stresses, which allow cellular homeostasis and contribute to plant survival (Kotak et al., 2007). Plant physiological processes such as photosynthesis will be highly affected by the rising global average temperature (Wahid et al., 2007; Saidi et al., 2011). Several cellular processes are affected by heat stress, which is particularly severe when high temperatures occur in conjunction with the critical stages of plant development, especially during the reproductive period (Teixeira et al., 2013). The effect of heat stress on the electron transport system, photosystems, pigments, photosynthesis-related enzyme activities, gas exchange, and chlorophyll fluorescence in plants have been examined by several researchers (e.g., Li et al., 2009; Mathur et al., 2011; Gan et al., 2014; Elferjani and Soolanayakanahally, 2018; Wang et al., 2021). Heat-stressed plant's chlorophylls and carotenoid contents undergo variations and an increased accumulation of photosynthetic products (carbohydrates) do occur (Zhou et al., 2020). Research conducted on wheat in Manhattan, USA, reports that carbon balance is influenced by heat stress, disrupting sink-source relations (Somayanda et al., 2019). When photosynthetic efficiency decreases under heat stress conditions due to the effects on membrane fluidity, decreased chlorophyll content, damage to mitochondria, reduced photochemical efficiency on photosystem II, and inactivation of Rubisco molecules, photoinhibition increases by triggering gene expression under high light intensity (Sharma et al., 2015; Huang et al., 2019). Plants show greater photosynthetic activity in summer months, correlated with plant growth and development; and photosynthetic assimilation production which is shared by various plant organs (Yasari et al., 2009; Egli & Bruening, 2003). In heat-stressed *B. napus*, chlorophyll content significantly decreased by 26.9 % under carbon-limited conditions and both photosynthesis and respiration rates dramatically decrease (Huang et al., 2019). Moreover, leaf photosynthesis and carbohydrate metabolism vary from genotype to genotype; this allows using this as a key indicator for the detection of heat susceptibility (Upchurch, 2008). Some examples of heat stress effects on plants are loss of biomass, reduced growth rate, altered development, and ultimately death (Somayanda et al., 2019; Yasari et al., 2009; Egli & Bruening, 2003). Heat stress can also cause protein denaturation, enhanced production of reactive oxygen species, and exert a negative influence on photosynthetic capacity resulting in metabolic imbalance (Cortleven et al., 2019; Timperio et al., 2008). Another important stress affecting photosynthesis is drought that can lead to various changes in plants' physiological activities including CO_2 assimilation, elongation of stem, leaf size reduction, reduced water use efficiency, or changes in chlorophyll content, stomatal conductance, and reduction in plant-water interaction (Razi & Muneer, 2021). Photosynthetic CO_2 assimilation, Rubisco, and stomatal conductance decreases in C4 plants when kept in water-deficit conditions (Carmo-Silva et al., 2010). This factor limiting photosynthetic conditions in C4 plants can be explained by ATP measurements, an inhibitor of RuBP that binds with Rubisco, and 3-phosphoglycerate of the Calvin cycle (Carmo-Silva et al., 2010).

1.4.3.1 Climate change and photosynthesis

According to the 2023 National Aeronautics and Space Administration (NASA) report, global temperature is repeatedly increasing with 2022 being the fifth warmest year since 1880. It has been predicted that global climate change will not only hurt valuable crop plants but also affect plants' ecological fitness (Ferguson, 2019). Global warming will trigger the evaporation and evapotranspiration of water sources leading to drought in arid and semi-arid regions; thus, overall water systems will be negatively influenced by climate change (Amanambu et al., 2020). A review by Ferguson (2019) showed that to develop more sustainable cropping systems, various research has been conducted on crop plants to cope with harsh environmental conditions like drought, flooding, and heat stress. Various studies showed that plants go through several changes due to climate change and one of the main issues of climate change is an elevated temperature that increases CO_2 content in the environment (Sage et al., 2008; Trivedi et al., 2022; Lan et al., 2022). Plants' major energy-producing process, which is photosynthesis, is highly affected by climate change. Heat and CO_2 increase due to climate change can lead to reduced plant growth because of reduced photosynthesis (Trivedi et al., 2022). The main enzyme in photosynthetic reactions (Rubisco) is highly sensitive to environmental changes like higher atmospheric CO_2 levels, and elevated temperature (Sage et al., 2008). There are disputed thoughts regarding how plants adapt to climate change, mainly regarding photosynthetic changes. These changes in plants that are due to the climatic adaptations may involve RuBP re-establishment by reducing electron transport capacity, or amplitude of Rubisco activase reductions for keeping Rubisco in proper form (Sage et al., 2008). The thermal optimal level of photosynthesis will increase along with global climate change and leaf temperature will increase (Sage et al., 2008). Overall, this implies that photosynthetic activity is a concern with changing climatic conditions (Dusenge et al., 2018). Climatic changes like temperature elevations (approximately 2-4 degrees by next century) and plants' exposure to this long-term temperature will result in plant's apparatus acclimation, or plants may stay stable by adaptations; but the long-term exposure may lead to naturally selecting the fittest plants that can survive under climatic changes (Sage et al., 2008).

1.4.4 Relationship between crop yield and photosynthetic efficiency

Plant yield and several other physiological and biological mechanisms have been reportedly found to be accelerated by the chlorophyll content (Miglani et al., 2021). Chlorophyll, one of the main components of photosynthesis, contains a high amount of chemical energy that helps in the assimilation of carbon creating a relationship between a mineral molecule and photosynthate administration (Miglani et al., 2021). Gang et al. (1992) reported that plant yield is positively correlated with chlorophyll content. The physiological mechanism behind this enhancement of productivity and yield by chlorophyll can be attributed to the enzyme, Rubisco involved in stomatal carbon fixation that utilizes ATP, a reaction assisted by chlorophyll pigment (Miglani et al., 2021). Additionally, non-photochemical quenching (NPQ) plays a crucial role in assessing photosynthetic efficiency, particularly in plants during negative growth stages, where increased efficiency may correlate with slower development (Friedland, 2019). In a rotational trial in Australia involving C3 (Wheat) and C4 (sorghum) plants, the level of stomatal conductance, CO_2 assimilation, and transpiration rates were high under optimum conditions indicating that the photosynthetic efficiency of these crops are higher (Wu et al., 2019). Consequently, in wheat and sorghum, these parameters enhancement showed higher yield (approximately 12.1%), indicating the importance of photosynthetically efficient plants (Wu et al., 2019). In plant leaves, the leaf dry mass per unit area (LMA) is a precursor of photosynthetic variation among species and LMA is higher in higher chlorophyll areas resulting in higher leaf thickness (Poorter et al., 2009; Ellsworth & Reich, 1993). When physiological parameters such as Rubisco capacity, electron transport rate, CO_2 assimilation rate, stomatal conductance, and different plant traits of several spring wheat genotypes were compared, Silva-Pérez et al. (2020) found significant genotypic variation where CO_2 assimilation rate and stomatal conductance showed the highest heritability (0.7 to 0.9). Thus, increased wheat photosynthetic efficiency and higher yield can be achieved by selecting superior germplasm for these traits, especially those showing high heritability (Silva-Pérez et al., 2020).

1.4.5 Plant morphological traits affecting photosynthesis metabolism

Plant morphological traits play a vital role in photosynthetic metabolism, influencing various physiological processes crucial for plant growth and development. Leaf area is an important morphological trait that can capture the photosynthetically active radiation. The higher the leaf area index (LAI), the greater the photosynthetic capacity (Gigova et al., 2013). Across various species and environments, metrics such as specific leaf area (SLA) and LAI coupled with gross assimilation rate (GAR), have emerged as important factors in explaining growth variations influenced by photosynthesis, particularly in response to different light intensities (Saleem et al., 2020). Stomata, the microscopic structures on the leaf epidermis, have significant control over gas exchange dynamics, regulating carbon dioxide for photosynthesis while concurrently managing water vapor release, thereby dictating carbon assimilation rates (Lawson & Blatt, 2014). Trichomes, which are hair-like structures on the leaf surface, serve as an important element for photosynthetic metabolism, modulating light penetration, boundary layer resistance, and temperature regulation; thus, exerting nuanced influences on photosynthetic performance (Wagner et al., 2004). In addition to trichomes, leaf surfaces are covered with waxes, which influence gas exchange patterns and water loss dynamics, thus shaping the photosynthetic landscape in response to environmental changes (Koch et al., 2006). Epidermal cells, forming the outermost layer of leaves, contribute to photosynthesis by fostering water retention and fortifying defenses against environmental stressors, ultimately nurturing an environment conducive to sustained photosynthetic activity (Schreiber, 2002).

1.5 Role and characterization of stomata

The evolution of stomata has been happening for over 400 million years with the changing condition of CO_2 level and moisture content (Franks & Farquhar, 2007). For the prediction of gas exchange regulation, stomatal morphology was found to be diversified functionally in four different species (*Heterospathe proliferans, Neottopteris exaltata, Thuja occidentalis, Triticum aestivum*) (Franks & Farquhar, 2007). Stomata can be modified for stressful environments like drought by the application of silicon that helps in photosynthetic efficiency, plant growth, and agronomic traits in sugarcane (Verma et al., 2020). A study in Saskatchewan, Canada showed that stomata in lentil germplasm stomata were more abundant on upper leaf surfaces, possibly for water conservation (Patel et al., 2021). Stomata are very sensitive plant organ whose opening and closing are highly dependent on environmental fluctuations (temperature rise or drop, water stress, light intensity) (Verma et al., 2020). Past research indicates that canola plants under water deficit conditions showed a lack of stomatal conductance, less leaf area, and leaf water content. Potassium application under drought conditions allowed increased stomatal conductance in canola leaves (Sharif et al., 2018; Waraich et al., 2020).

1.6 Role and characterization of trichomes

Plant trichomes serve as a crucial defense mechanism against insect pests, and high trichome density in plants can help deter herbivores (War et al., 2012). In North America, integrated pest management has become an important topic due to flea beetle infestation in canola and canola cultivars with more trichomes tend to be more resistant to insects (Gavloski, 2017). In a study conducted in Saskatoon and Lethbridge involving transgenic canola lines with more trichomes, they were less susceptible to flea beetles in comparison to parent canola lines with fewer trichomes (Soroka et al., 2007). Flea beetle feeding was reduced by 30-50 % in two *B. napus* lines with hairy cotyledons and hairy true leaves (Alahakoon et al., 2016). Trichome acts as a defense mechanism in *B. rapa* when kept under elevated CO_2 concentration and showed a 57 % increase in trichome density (Karowe & Grubb, 2011). However, most canola cultivars lack trichomes. Researchers are trying to introduce trichomes from other Brassica species, like *Brassica villosa* which carry hairy outgrowths that accumulates metals and alkaloid-like compounds and make the plant resistant to disease and insect (Nayidu et al., 2015). Trichomes are crucial for plant defense against climate-related stresses. A study on lentil germplasm examined trichromes in 12 genotypes and showed the presence of significant variation in trichome density and length; this study also revealed the quantitative nature of the trichome trait, providing insights for breeding resilient lentil cultivars (Patel et al., 2021). Although glandular and non-glandular trichomes are commonly found, most *Brassica* species assessed so far contain unbranched non-glandular trichomes (Soroka et al., 2011).

1.7 Rationale

Source-sink relationship, studied for a long time in the field of plant science in various crops is the underlying mechanism governing biomass allocation and partitioning. Photosynthesis is the main process behind source-sink mechanism, and important in determining if crop yields are limited to source or sink activity. Several research findings have shown that the yield is linked to source-sink dynamics in all crops (Faraji, 2014; Fatichi et al., 2014; Parry et al., 2013; Zhang & Flottmann, 2018). Currently, breeders and agronomists have increased canola production through the development of genetically improved cultivars and the implementation of improved cultural practices at the farm level (Morrison et al. 2016). To further increase the yield of canola cultivars, breeders need to incorporate alternate strategies, such as increasing the photosynthetic efficiency (PE) of the cultivars; this strategy has been proposed by the wheat consortium to increase wheat yields (Parry et al., 2011). To our knowledge, only one greenhouse experiment on PE has been conducted using a single canola cultivar (Elferjani and Soolanayakanahally, 2018). No studies have been conducted to study source-sink dynamics by assessing biomass partitioning and allocation under field conditions in genetically diverse canola accessions and cultivars to exploit this physiological trait in breeding programs. This study involves an integration of climatic factors, photosynthesis, and breeding; this is the first of its kind in the prairies and is unique in that it serves as a pioneer experiment and model for similar research on other crops. This research assessed 168 canola accessions from seven different pedigree groups to identify canola accessions with superior biomass and resource partitioning and characterize leaf morphological structures (trichomes and stomata) in these canola accessions.

1.7.1 Objectives

The objectives of this project are:

1. To assess the differences in biomass partitioning and allocation canola accessions belonging to different pedigree groups.
2. To characterize the leaf anatomical structures of different canola accessions

1.7.2 Null hypotheses

I. Canola accessions from different parental backgrounds partition their resources in a similar manner.

II. There are no differences in leaf anatomical structures between different canola accessions.

Chapter 2 Source-sink relationship and resource partitioning in different canola accessions

2.1. Introduction

Canola, a member of the Brassicaceae family, commonly known as rapeseed, is grown for its oil-rich seeds. Canola shows resilience in drier regions when compared with other Brassicaceae species, as evidenced by studies comparing its yield potential with that of linola and Indian mustard in Australia (Hocking et al., 1997). Despite its adaptability, the quest for higher-yielding canola persists due to observed yield losses and challenges under field conditions (Haile et al., 2014). The Canadian canola industry is actively pursuing various approaches to overcome these obstacles (Rampel et al., 2014). Understanding resource (biomass) allocations in canola is pivotal for enhancing its productivity. The concept of resource allocation involves the distribution of resources produced during the process of photosynthesis from source organs, to sink organs, which utilize these resources for growth and development. Through the photosynthesis process plants produce photoassimilates which are regulated through the source and sink organs - source is the producer and sink is the utilizer of photoassimilates (Ho, 1988). Sink organs rely on imported photosynthate, that is a carbon based compound (sugar or other related form), for growth and metabolism (Ho, 1988). Mature leaves act as net exporter of photosynthate, supplying sink organs and enabling growth (Ludewig & Sonnewald, 2016). Several researchers postulate that the main physiological factors directly influencing plant growth include i) photosynthetic efficiency (Ort et al., 2015) and ii) source-sink effects (Jonik et al., 2012; Rossi et al., 2015; Sweetlove et al., 1998). Improvement of crop photosynthetic efficiency is one of the most important areas for the enhancement of yield potential (Long et al., 2006). This efficiency is determined by different factors such as the plant's ability to capture and utilize sunlight, convert carbon dioxide into sugars through photosynthesis, and allocate these sugars to different plant parts for growth and development (Ludewig & Sonnewald, 2016). As the source organs (mature leaves) captures sunlight, understanding their LAI, which represents the amount of photosynthetically active leaf area available for light interception and assimilation, is crucial as it directly influences photosynthetic activity contributing to enhanced plant growth (Lindroth et al., 2008). With increasing plant density, LAI increases and the increased LAI results in greater yield potential of crop plants (Zhang et al., 2021). Efficient partitioning ensures that each organ receives the necessary resources for optimal growth and function, ultimately contributing to higher yields (Rivera-Amado et al., 2019). Therefore, understanding the mechanisms underlying dry matter partitioning is essential for maximizing crop productivity (Rivera-Amado et al., 2019).

The flow of assimilates from source to sink organ results in dry matter partitioning (Marcelis, 1996). Dry matter partitioning, which involves the distribution of total biomass among leaves, stems, roots, flowers, and seeds throughout the plant's growth and development, is integral to this understanding. Bhattacharya (2022) underscores the importance of understanding how carbon assimilates are partitioned among the plant's source and sink organs for overall crop yield. Efficient allocation of carbon to different plant parts ensures balanced growth, enabling adaptive changes in response to resource availability - a characteristic unique to plants (Bhattacharya, 2022). Variability in dry matter partitioning among rice cultivars further elucidates its impact on autotrophic growth and overall crop production (Asch et al., 1999). The understanding the relationship of plants total biomass that is divided into root, stem and leaf is simplified by the ratio of the root to shoot (total of stem and leaf biomass) biomass partitioning (Mokany et al., 2006). Root/shoot ratio has been used to assess aboveground and belowground carbon and nitrogen distribution in Canadian canola (Thiagarajan et al., 2018). Generally, directing a high proportion of dry matter to seeds is essential for maximizing yield and oil content in canola, given that the developing seeds serve as the major sink organ. Studies by Arvin et al. (2014) highlight the positive correlation between silique dry matter partitioning and yield, emphasizing the importance of efficient resource allocation. Yield differences were attributed to variations in biomass, nitrogen uptake, and sink-strength during post-anthesis, where cultivars exhibited different strategies for increased seed yield, either by increasing grain number or by enhancing grain size, indicating a trade-off between fruiting efficiency and grain weight in durum wheat (Ferrante et al., 2012). Furthermore, research on the relationship between harvest index and dry matter partitioning underscores the significance of balanced nutrient application, as demonstrated in rice cultivation (Arvin et al., 2014; Amanullah & Inamullah, 2016). To enhance crop yield, it is essential to address the obstacles such as adverse environmental conditions, poor agronomic practices, and various diseases. While measures like field covers, irrigation, and pesticide use mitigate environmental challenges, less attention is given to plant intrinsic factors like nutrient allocation, particularly carbon allocation between sources and sink tissue (Ludewig & Sonnewald, 2016). Thus, understanding the intricate interplay between source-sink dynamics and resource partitioning holds promise for optimizing canola productivity amidst changing environmental conditions. This relationship between source and sink organs dictates the flow of resources within the plant, influencing growth, development, and ultimately yield. Consequently, elucidating the mechanisms governing these processes is essential for breeding programs aiming to enhance canola resilience and productivity.

2.1. Materials and Methods

2.2.1. Plant Material

A total of 168 *B. napus* accessions and two check cultivars, viz., L255PC (Liberty Link) and 45H33 (Roundup Ready) were evaluated in this study. The 168 accessions derived from seven different backgrounds including the (winter canola × spring canola) × rutabaga ((W × S) × R), spring canola × *Brassica oleracea* (S × Oleracea), spring canola × rutabaga (S × R), spring canola × *Brassica rapa* (S × Rapa) , spring canola × spring canola (S × S), and winter canola × spring canola (W × S) crosses, as well as accessions collected from The Plant Gene Resources of Canada, Saskatoon (Gene bank).

**See appendices supplementary table 2.1 for the list of canola accessions assessed.*

2.2.2. Site Description

This experiment was conducted for three seasons (2021 to 2023) located in the black soil zones at three different sites in Alberta, viz., West-240 (South campus, University of Alberta; 2021, 2023), St. Albert research station (2022, 2023), and Crop diversification center (CDC) North (2023). The black chernozemic soil was 12 to 20 cm thick and contain 6-10% organic matter (https://soil.agric.gov.ab.ca/agrasidviewer/). Weather data including precipitation, temperature, and vapor pressure deficit was gathered from the nearest weather stations during the crop growing season (https://climate.weather.gc.ca).

2.2.3. Experimental Design

The experimental design was an alpha lattice with three replications. To accommodate the 168 accessions in best possible way, each replication was divided into six blocks, and each block included 28 accessions and two checks. Thus, the total number of blocks was 18 and the total number of plots per site was 540 (30 entries including checks × 6 blocks × 3 replications). Plot size was 5 m x 1.8 m (L × W). Seeds were treated with Halex® GT and Lumiderm™ to control flea beetles and cutworms. Fertilizer (11.52.0) was broadcasted at all sites at 30lb/acre before seeding based on soil test. Before seeding, pre-emergent herbicide Edge® MicroActiv® was applied at 12.5 lb/acre to control broadleaf weeds. Seeding was done with a four-row seeder Fabro (Swift Current) and targeted number seeds/m^2 was 140. To control the grassy weed and Canada thistle, Poast® Ultra (300ml/acre) and Lontrel™ XC (250ml/acre) with Merge® adjuvant were applied at four-leaf stage. In 2023, the west-240 site was heavily infested with lygus bugs at the time of flowering; to control this insect, decis® 5 EC insecticide (60ml/acre) was sprayed. When the crop was 90 % mature (BBCH 89), Reglone® Ion – desiccant (1.3L/acre) was sprayed to desiccate the crop. Eight days post application; the plots were harvested with a Wintersteiger plot combine (model-Delta) equipped with an automatic weighing system.

2.2.3. Data Collection

2.2.3.1. Agronomic and plot establishment traits

Agronomic and crop establishment data collected include plant density, days to flowering and plant height. For plant density, seedlings at the cotyledon stage (BBCH 10) were counted in one meter of the front and back of each plot and plant density calculated using the following formula-

$$Plant\ Density\ (plants\ per\ unit\ area) = \frac{Number\ of\ emerged\ plants}{Area\ of\ study\ plot}$$

Days to flowering was recorded when about 50% of the plants in a plot had 20+ flowers on the main stem at BBCH 65. Flowering data was recorded from all sites in 2023 only. Plant height was recorded at BBCH 75, i.e. when about 50% of the siliques on a plant turned to yellow color.

2.2.3.2. Measurement of physiological, biomass and yield traits

The following traits were recorded: LAI, plant biomass (dry weight), seed yield, and harvest index (HI). LAI is the amount of leaf per meter squared and was measured at BBCH 50 (flower buds present but nor opened yet) below the canopy with an AccuPAR LP-80 ceptometer, manufactured by Decagon Devices (now METER Group, Inc.), also designed to measure photosynthetically active radiation (PAR) above and below a plant canopy. This data helps estimate the amount of light energy absorbed by the canopy. For plant biomass, five plants were randomly selected from each plot at BBCH 50 and were uprooted ensuring that the entire root system is intact. Root, stem and leaves were separated, and the stems and leaves were placed in paper bags for drying. The roots were kept in a cooler, later washed and placed in paper bags for drying. The samples were oven dried at 65 °C for four days. Leaf, stem and roots dry weights were measured using a Denver instrument SI-234 summit series analytical balance (Denver instruments, USA). Based on this, root/shoot (R/S) ratio was calculated using the following formula:

$$Root/\ shoot\ ratio = \frac{Root\ dry\ weight}{(Leaf\ dry\ weight + Stem\ dry\ weight)}$$

The plot combine automatically measures the total seed weights per plot and the data converted to kg/ha yield. One thousand seeds from each plot were counted and weighed with a SI-234 balance for 1000-seed weight (TKW) determination.

Based on seed yield and biomass data, harvest index (HI) was calculated using the following formula:

$$Harvest\ Index\ (HI) = \frac{Yield}{Total\ aboveground\ biomass} \times 100$$

2.2.3.5. Statistical Analysis

All statistical analyses were done with R version 4.1.1 (R Core Team, 2021). The dataset of the 168 canola accessions was grouped into seven groups based on their pedigree groups or origin. The data was tested for normality with qqplots (Wickham, 2016) and Shapiro-Wilk test using the base functions of R and all data were found to be normally distributed. The model, fitted using the lmer function in R (R Core Team, 2021), investigated the individual and interactive effect of the canola accessions and experimental sites in each pedigree group. The dataset from all five sites was analyzed. For analysis of variance (ANOVA), the canola accessions were considered as fixed effect and replication, and block were considered as random effects. A linear mixed-effects model was fitted to the data using the lmer function of the lme4 package (Bates et al., 2015). Estimated marginal means were calculated using the emmeans function from the emmeans package (Lenth, 2021) for pairwise comparisons of the lines factor. Multiple comparison adjustments were performed using the cld function from the multcomp package (Horthorn et al., 2008). This approach allowed us to assess the combined effect of both factors' sites, and different canola lines, and their interaction effects at $P<0.001$, $P<0.01$, and $P<0.05$ levels of significance (Table 2.1). The violin plots were drawn to show the median, quartiles, and potential outliers within each distribution. These plots were generated using the ggplot2 package in R. Each violin plot represents the distribution of a continuous variable ("Leaf," "Total Biomass," "Stem," "Root," "R/S," "Yield," "TKW," "HI," or "LAI") across different experimental sites ("Sites") for each pedigree groups. To determine significant differences between the experimental sites for each variable, a Tukey test was performed using the HSD.test function from the agricolae package (De Mendiburu & Yaseen, 2020)The letters for grouping were added to the violin plots to indicate statistically significant differences at $P<0.05$ level. Boxplots were generated to compare different sites within each canola accessions, aiming to elucidate the interaction effects on yield and harvest index (HI) in the dataset. Grouped boxplots were created using the ggplot2 (Wickham, 2016) package, to visualize the LAI for different canola pedigree groups at different site years. The ggpubr (Kassambara, 2020) package was used to arrange multiple plots into a single figure. Emmeans comparisons were done to identify the canola accession that outperformed the checks. Principal component analysis was also conducted to identify the variables with significant effects and the relationship within the variables. PCA was performed using the FactoMineR package (Lê et al., 2008). Principal Component Analysis (PCA) serves as a valuable method for analyzing multivariate data, facilitating the categorization of experimental units into groups based on similarity. The analysis included 170 (168 +2 checks) canola accessions and eight response variables, including biomass (total biomass, leaf, stem, root, R/S), seed yield, LAI, and TKW at five site years. The resulting biplot, incorporating PC scores and eigenvectors, aids in identifying variables strongly associated with specific principal components (PCs). Eigenvalues were visualized using the fviz_eig() function of the factoextra package (Kassambara & Mundt, 2020). Various aspects of the principal components, including coordinates, quality on the factor map (Cos2), and contributions to the principal components, were extracted using appropriate functions. Principal components were visualized using functions like fviz_pcavar () and corrplot. Pearson's correlation coefficients were calculated using specific models within RStudio.

2.3. Results

2.3.1. Weather conditions

In 2021, the West- 240 sites experienced drought conditions during the growing season with cumulative rainfall < 200 mm which was lower than average precipitation, higher temperatures at this site, evidence of a dry year (Figure 2.1, a). In 2022 and 2023 had average rainfall (Figure 2.1, a). In 2021 and 2022, the average temperatures were approx. 10 degrees at the beginning of the growing season; however, a sharp increase was seen by July (Figure 2.1, b). In 2023, all three sites had average temperature of about 15°C to 19°C, and temperature fluctuation was not noteworthy at this time (June to August) (Figure 2.1, b). Vapor pressure deficit (VPD) had a sharp increase at the West-240 site in June 2021 (Figure 2.1, c). High VPD indicates low humidity or high temperatures when atmospheric demand for moisture become high, and this can result in increased water loss by the plants. Thus, the crop at West240 site in 2021 suffered from water stress; the rest of the sites in 2022 and 2023 had average VPD.

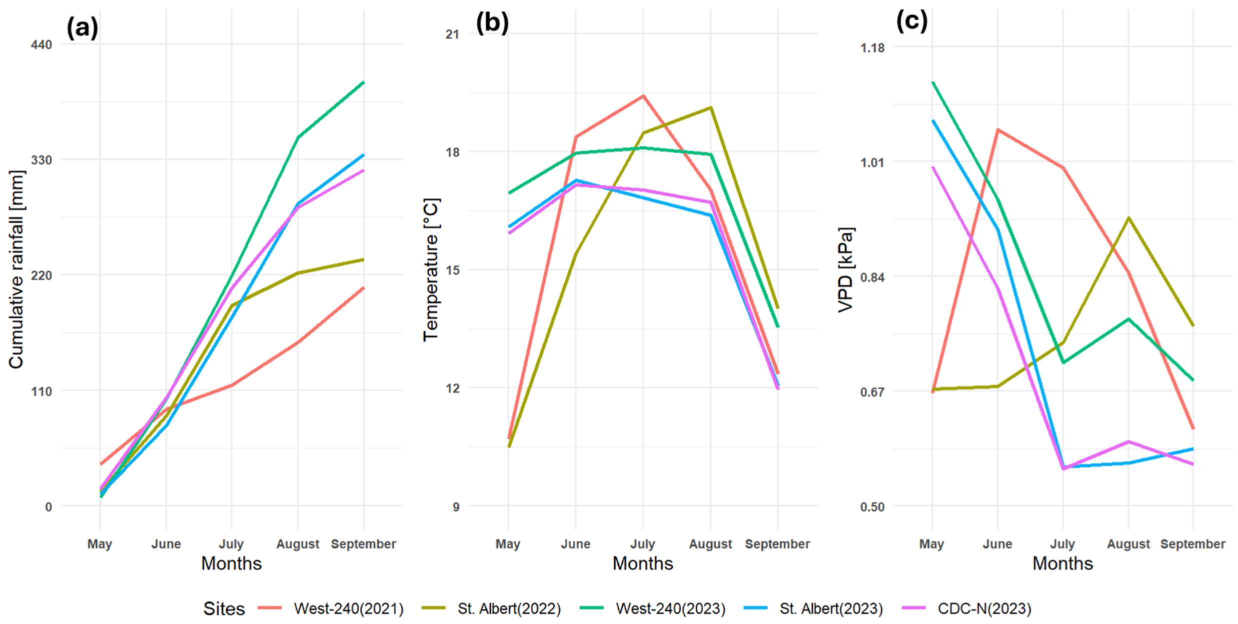

Figure 2. 1 Line graph presenting monthly mean of (a) cumulative rainfall, (b) average temperature, and (c) vapor pressure deficit (VPD) during the growing season (May to September) at different site and years. [Data source: https://climate.weather.gc.ca]

2.3.2. Characterization of the canola accessions for agronomic traits

Plant density, plant height, days to flowering, and days to maturity data were taken to characterize the populations for these basic agronomic traits. There was no visible difference in plant density between the canola pedigrees groups and none of the pedigree groups were significantly different from the checks. However, difference for plant density was found between the five sites in three years. High canola density was seen in 2021 at West-240 site where the check L255PC had a higher mean value and the pedigree group (W × S) × R was the closest to the check. In 2023, West-240 and St. Albert sites had lower plant density than the other three sites (Figure 2.2, a). Plant density can influence resource utilization by creating more competition if the density is higher and less competition for resources if the plant density is lower. However, we did not anticipate this to happen considering the ability of canola to produce more branches and offset any effects at low seeding densities. Plant height data was taken only in 2022 and 2023. At all three sites in 2023, height of the plants of all pedigree groups were similar and was taller than the plants at St. Albert site in 2022 (Figure 2.2, b). Taller canola plants are more susceptible to lodging, which occurs when the stem strength is unable to support the weight of the canopy. Lodging can disrupt the source-sink relationship by reducing the availability of light to the leaves, affecting photosynthetic activity, and potentially by limiting the production of assimilates and supply to the sinks. Mean height of the seven pedigree groups was not significantly different and they were similar to the checks. Days to flowering (DTF) and days to maturity (DTM) data were taken only in 2023 from three sites. The mean values of the West-240 site were higher than the other two sites for both DTF and DTM, indicating that canola flowered as well as matured late at this site (Figure 2.2, (c), (d)). On the other hand, the St Albert site flowered early but matured later, due to later seeding at this site compared to the other two sites. On the Canadian prairies, the growing season is relatively short; therefore, a knowledge of DTF and DTM is important for producers to avoid frost damage.

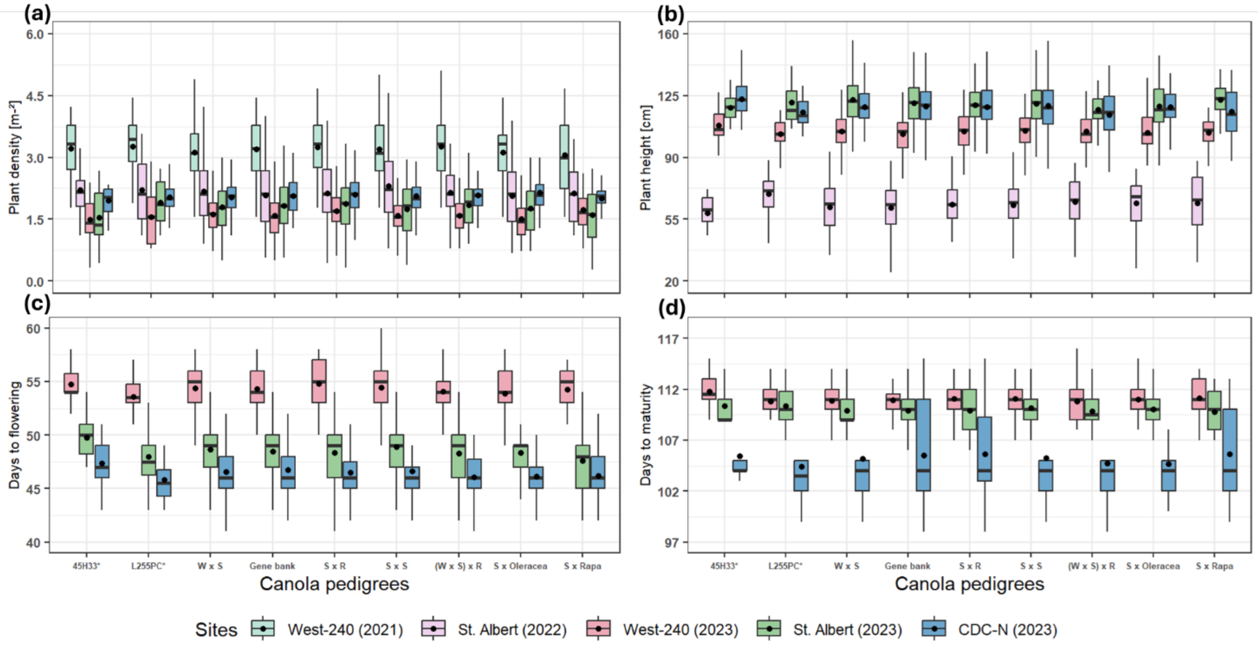

Figure 2. 2 Boxplots presenting (a) plant density, (b) plant height, (c) days to flowering, and (d) days to maturity of different canola pedigree groups at various site years. Points in the box indicate the mean and the horizontal bars indicate the median value.[(winter canola × spring canola) × rutabaga ((W × S) × R), spring canola × *Brassica oleracea* (S × Oleracea), spring canola × rutabaga (S × R), spring canola × *Brassica rapa* (S × Rapa) , spring canola × spring canola (S × S), and winter canola × spring canola (W × S) crosses, as well as accessions collected from The Plant Gene Resources of Canada, Saskatoon (Gene bank)]. * Indicates Checks [L255PC (LibertyLink) & 45H33 (Roundup Ready)].

2.3.3. Characterization of the canola accessions for physiological, biomass and yield traits

Analysis of variance indicated that the trial sites exerted significant effect on all physiological traits, viz. total biomass, leaf biomass, stem biomass, root biomass, R/S ratio, TKW, yield, HI, and LAI (Table 2.1). Interaction of the accessions and sites was significant for yield and HI for the S × Oleracea pedigree group. A significant difference within the canola accessions was found in most of the pedigree groups for different traits, such as in S × Oleracea for TKW, in S × R for yield, in S × S for R/S and yield, in W × S for root biomass, R/S ratio, TKW, and yield in

Gene Bank accessions for yield and TKW (Table 2.1). When testing canola pedigree groups for the significant difference between sites, a significant variation was found for all variables assessed. On average, LAI at West-240 (2023), St. Albert (2023) and CDC-North (2023) site, none of the canola pedigree groups outperformed the checks (Figure 2.3). Each canola pedigree group has different number of canola accessions. Therefore, biomass allocations, yield and yield related traits were discussed for each pedigree group.

In (W × S) × R pedigree group, total biomass at CDC-North (2023) site was significantly higher (p<0.5) than the rest of the sites; West-240 (2023) had the lowest total biomass (Figure 2.4, a). Leaf biomass at CDC-North (2023) was significantly higher than other sites while West-240 (2021) and West-240 (2023) had the lowest leaf biomass (Figure 2.4, b). Stem biomass at CDC-North (2023) was significantly higher compared to the other four sites; West-240 (2023) had the lowest stem biomass (Figure 2.4, c). Root biomass was significantly higher at CDC-North (2023) than the other sites (Figure 2.4, d). Significantly higher R/S ratio was observed at West-240 (2023) and West-240 (2021) while significantly low R/S ratio was found in St. Albert (2022) and CDC-North (2023) (Figure 2.4, e). TKW was significantly higher at CDC-North (2023) and (West-240 (2023) (Figure 2.4, f). Yield was significantly higher at CDC-North (2023) and significantly low at West 240 (2021); St. Albert (2022), West-240 (2023) and St. Albert (2023) had similar yields (Figure 2.4, g). HI was significantly higher at West-240 (2023); the rest of the sites had similar HI (Figure 2.4, h).

In the S × Oleracea pedigree group, total biomass at St. Albert (2023) and CDC-North (2023) were significantly higher (p<0.5) than the rest of the other sites (Figure 2.5, a). Leaf biomass at CDC-North (2023) and St. Albert (2023) was significantly higher than at other sites, West-240 (2021& 2023) had significantly low leaf biomass (Figure 2.5, b). Stem biomass at CDC-North

(2023) was significantly higher; West-240 (2021), St Albert (2022), West-240 (2023) had the lowest stem biomass (Figure 2.5, c). Root biomass was significantly higher at CDC-North (2023) and St. Albert (2023) than the other sites (Figure 2.5, d). Significantly higher R/S ratio was observed at West-240 (2023) and West-240 (2021) than the rest of the sites (Figure 2.5, e). TKW was significantly higher at CDC-North (2023) and (West-240 (2023) (Figure 2.5, f). Yield was significantly higher at CDC-North (2023) and St. Albert (2023); St. Albert (2022) and West-240 (2021) had lowest yield (Figure 2.5, g). HI was significantly higher at West-240 (2023); the rest of the sites had similar HI (Figure 2.5, h).

In the S × R pedigree group, total biomass at CDC-North (2023) was significantly higher ($p<0.5$) than the rest of the sites; West-240 (2023) and West-240 (2021) were significantly lower (Figure 2.6, a). Leaf biomass at CDC-North (2023) was significantly higher than other sites while West-240 (2021) and West-240 (2023) had the lowest leaf biomass (Figure 2.6, b). Stem biomass at CDC-North (2023) was significantly higher; West-240 (2023) had the lowest stem biomass (Figure 2.6, c). Root biomass was significantly higher at CDC-North (2023) than the other sites (Figure 2.6, d). Significantly higher R/S ratio was observed at West-240 (2023) and St. Albert (2023) (Figure 2.6, e). TKW was significantly higher at CDC-North (2023) and West-240 (2023) (Figure 2.6, f). Yield was significantly higher at CDC-North (2023) and St. Albert (2023); West-240 (2021) had the lowest yield (Figure 2.6, g). HI was significantly higher at West-240 (2023); the rest of the sites had similar HI(Figure 2.6, h).

In the S × S pedigree group, total biomass at CDC-North (2023) was significantly higher ($p<0.5$) than the rest of the sites; West-240 (2023) and West-240 (2021) were the lowest total biomass (Figure 2.7, a). Leaf biomass at CDC-North (2023) was significantly higher than other sites; West-240 (2021) had the lowest leaf biomass (Figure 2.7, b). Stem biomass at CDC-North

(2023) was significantly higher; West-240 (2023) had the lowest stem biomass (Figure 2.7, c). Root biomass was significantly higher at CDC-North (2023) than at the other sites (Figure 2.7, d). Significantly higher R/S ratio was observed at West-240 (2023) and St. Albert (2023) (Figure 2.7, e). TKW was significantly higher at CDC-North (2023) and (West-240 (2023) (Figure 2.7, f). Yield was significantly higher at CDC-North (2023) and St. Albert (2023); West-240 (2021) had the lowest yield (Figure 2.7, g). HI was significantly higher at West-240 (2023) than the rest of the sites and CDC-North (2023) had the lowest HI (Figure 2.7, h).

In the S × Rapa pedigree group, the total biomass at CDC-North (2023) was significantly higher ($p<0.5$) than the rest of the sites; West-240 (2023), West-240 (2021) and St. Albert (2022) had the lowest total biomass (Figure 2.8, a). Leaf biomass at CDC-North (2023) was significantly higher than other sites; West-240 (2021) and West-240 (2023) had the lowest leaf biomass (Figure 2.8, b). Stem biomass at CDC-North (2023) was significantly higher; West-240 (2023) had the lowest stem biomass (Figure 2.8, c). Root biomass was significantly higher at CDC-North (2023) than the other sites (Figure 2.8, d). Significantly higher R/S ratio was observed at West-240 (2021), St. Albert (2022), West-240 (2023) and St. Albert (2023) (Figure 2.8, e). TKW was significantly higher at CDC-North (2023) and (West-240 (2023) (Figure 2.8, f). Yield was significantly higher at CDC-North (2023) and St. Albert (2023); West-240 (2021) had the lowest yield (Figure 2.8, g). HI was significantly higher at West-240 (2023) than the rest of the sites (Figure 2.8, h).

In the W × S pedigree group, total biomass at CDC-North (2023) was significantly higher ($p<0.5$) than the rest of the sites; West-240 (2023), West-240 (2021) and St. Albert (2022) had significantly lower total biomass (Figure 2.9, a). Leaf biomass at CDC-North (2023) was significantly higher than other sites; West-240 (2021) and West-240 (2023) had the lowest leaf biomass (Figure 2.9, b). Stem biomass at CDC-North (2023) was significantly higher; West-240

(2023) had the lowest stem biomass (Figure 2.9, c). Root biomass was significantly higher at CDC-North (2023) and St. Albert (2023) (Figure 2.9, d). Significantly higher R/S ratio was observed in West-240 (2021), West-240 (2023) and St. Albert (2023) (Figure 2.9, e). TKW was significantly higher at CDC-North (2023) and West-240 (2023) (Figure 2.9, f). Yield was significantly higher at CDC-North (2023) and St. Albert (2023); West-240 (2021) had the lowest yield (Figure 2.9, g). HI was significantly higher at West-240 (2023) (Figure 2.9, h).

In the Gene Bank pedigree group, total biomass at CDC-North (2023) was significantly higher ($p<0.5$) than the rest of the sites; West-240 (2023) and West-240 (2021) had the lowest total biomass (Figure 2.10, a). Leaf biomass at CDC-North (2023) was significantly higher than other sites; West-240 (2021) and West-240 (2023) had the lowest leaf biomass (Figure 2.10, b). Stem biomass at CDC-North (2023) was significantly higher; West-240 (2023) had the lowest stem biomass (Figure 2.10, c). Root biomass was significantly higher at CDC-North (2023) (Figure 2.10, d). Significantly higher R/S ratio was observed at West-240 (2021) and West-240 (2023) (Figure 2.10, e). TKW was significantly higher at CDC-North (2023) and West-240 (2023) (Figure 2.10, f). Yield was significantly higher at CDC-North (2023) and St. Albert (2023); West-240 (2021) had the lowest yield (Figure 2.10, g). HI was significantly higher at West-240 (2023) compared to other sites; CDC-North (2023) had the lowest HI (Figure 2.10, h).

Significant accessions × site interaction for seed yield and HI was found for the S × Oleracea pedigree group (Table 2.1). Yield of the canola accessions belonging to this group ranged from 3000 kg/ha to 6000 kg/ha at the CDC-N (2023) and St. Albert (2023) sites (Figure 2.11). The greatest yield was observed for the canola accession 5CA1679 801-A2099 at both CDC-N (2023) and St. Albert (2023) sites (Figure 2.11). The lowest seed yield for all accessions was recorded from the West-240 (2021) site, where yield ranged from 2000 kg/ha to 3000kg/ha. HI was similar at all site years for all canola pedigree groups (Figure 2.12). Significantly high (*p*<0.5) HI compared to the checks was observed in the canola accessions, 5CA1679 801-A2099 at CDC-N (2023) and St. Albert (2023), and 5CA1679 825-A2090 at West-240 (2021 and 2023). The genotype-by-environment interaction showed that the canola accessions performed differently across the different sites.

Table 2. 1 Analysis of variance showing the individual and interactive effects of the canola lines and sites for different pedigree groups for plant total biomass (TDwt), leaf biomass (LDwt), stem biomass (SDwt), root biomass (RDwt), root/shoot biomass ratio [R/S], thousand seed weight [TKW], yield, harvest index [HI], and leaf area index [LAI]. ***, **, and * represents significance at 0.001,0.01 and 0.05 probability levels. ns means non-significant.

Canola pedigree groups	Sources of variation	TDwt	LDwt	SDwt	RDwt	R/S	TKW	Yield	HI	LAI
(W × S) × R	Accessions (A)	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Sites (S)	<0.00***	<0.00***	<0.00***	<0.00***	<0.00***	<0.00***	<0.00***	<0.00***	<0.00***
	A× S	ns	ns	ns	ns	ns	ns	ns	ns	ns
S × Oleracea	Accessions (A)	ns	ns	ns	ns	ns	0.02*	ns	ns	ns
	Sites (S)	<0.00***	<0.00***	<0.00***	<0.00***	<0.00***	<0.00***	<0.00***	<0.00***	<0.00***
	A × S	ns	ns	ns	ns	ns	ns	0.04*	0.008**	ns
S × R	Accessions (A)	ns	ns	ns	ns	ns	ns	0.02*	ns	ns
	Sites (S)	<0.00***	<0.00***	<0.00***	<0.00***	<0.00***	<0.00***	<0.00***	<0.00***	<0.00***
	A × S	ns	ns	ns	ns	ns	ns	ns	ns	ns
S × S	Accessions (A)	ns	ns	ns	ns	0.01*	ns	0.005**	ns	ns
	Sites (S)	<0.00***	<0.00***	<0.00***	<0.00***	<0.00***	<0.00***	<0.00***	<0.00***	<0.00***
	A × S	ns	ns	ns	ns	ns	ns	ns	ns	ns
S × Rapa	Accessions (A)	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Sites (S)	<0.00***	<0.00***	<0.00***	<0.00***	<0.00***	<0.00***	<0.00***	<0.00***	<0.00***
	A × S	ns	ns	ns	ns	ns	ns	ns	ns	ns
W × S	Accessions (A)	ns	ns	ns	0.01*	0.03*	0.008**	0.03*	ns	ns
	Sites (S)	<0.00***	<0.00***	<0.00***	<0.00***	<0.00***	<0.00***	<0.00***	<0.00***	<0.00***
	A × S	ns	ns	ns	ns	ns	ns	ns	ns	ns
Gene bank	Accessions (A)	ns	ns	ns	ns	ns	0.03*	0.04**	ns	ns
	Sites (S)	<0.00***	<0.00***	<0.00***	<0.00***	<0.00***	<0.00***	<0.00***	<0.00***	<0.00***
	A × S	ns	ns	ns	ns	ns	ns	ns	ns	ns

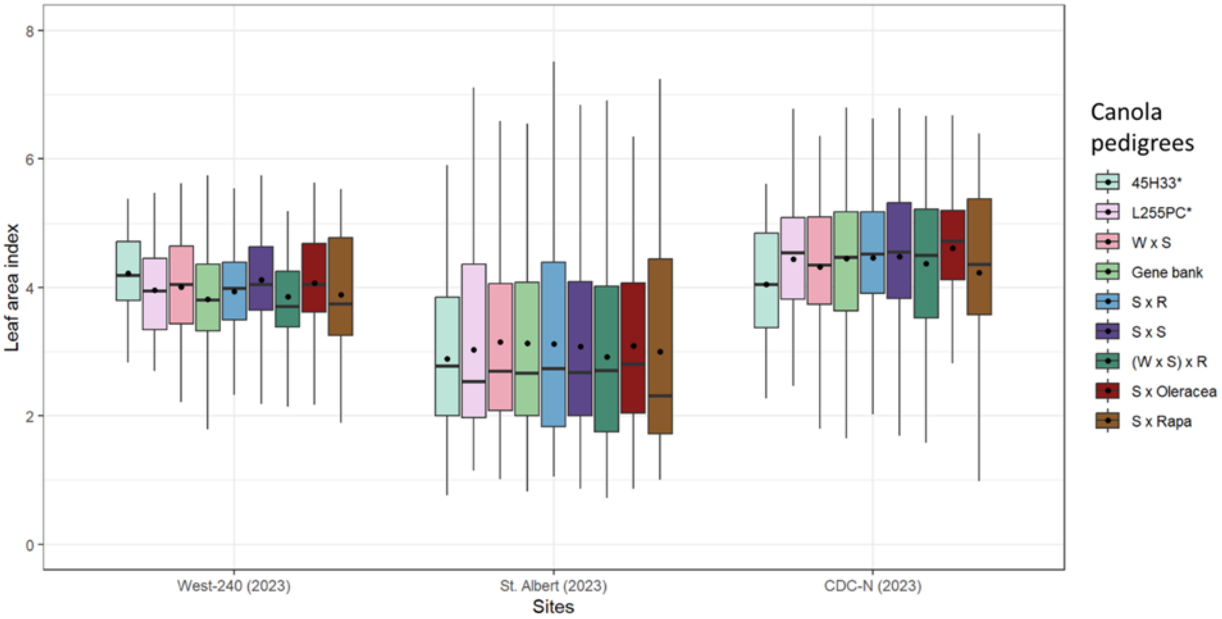

Figure 2. 3 Boxplots presenting leaf area index of different canola pedigree groups at three site years. Points in the box indicate the mean and the horizontal bars indicate the median values. [(winter canola × spring canola) × rutabaga ((W × S) × R), spring canola × *Brassica oleracea* (S × Oleracea), spring canola × rutabaga (S × R), spring canola × *Brassica rapa* (S × Rapa), spring canola × spring canola (S × S), and winter canola × spring canola (W × S) crosses, as well as accessions collected from The Plant Gene Resources of Canada, Saskatoon (Gene bank)]. * Indicates Checks [L255PC (LibertyLink) & 45H33 (Roundup Ready)].

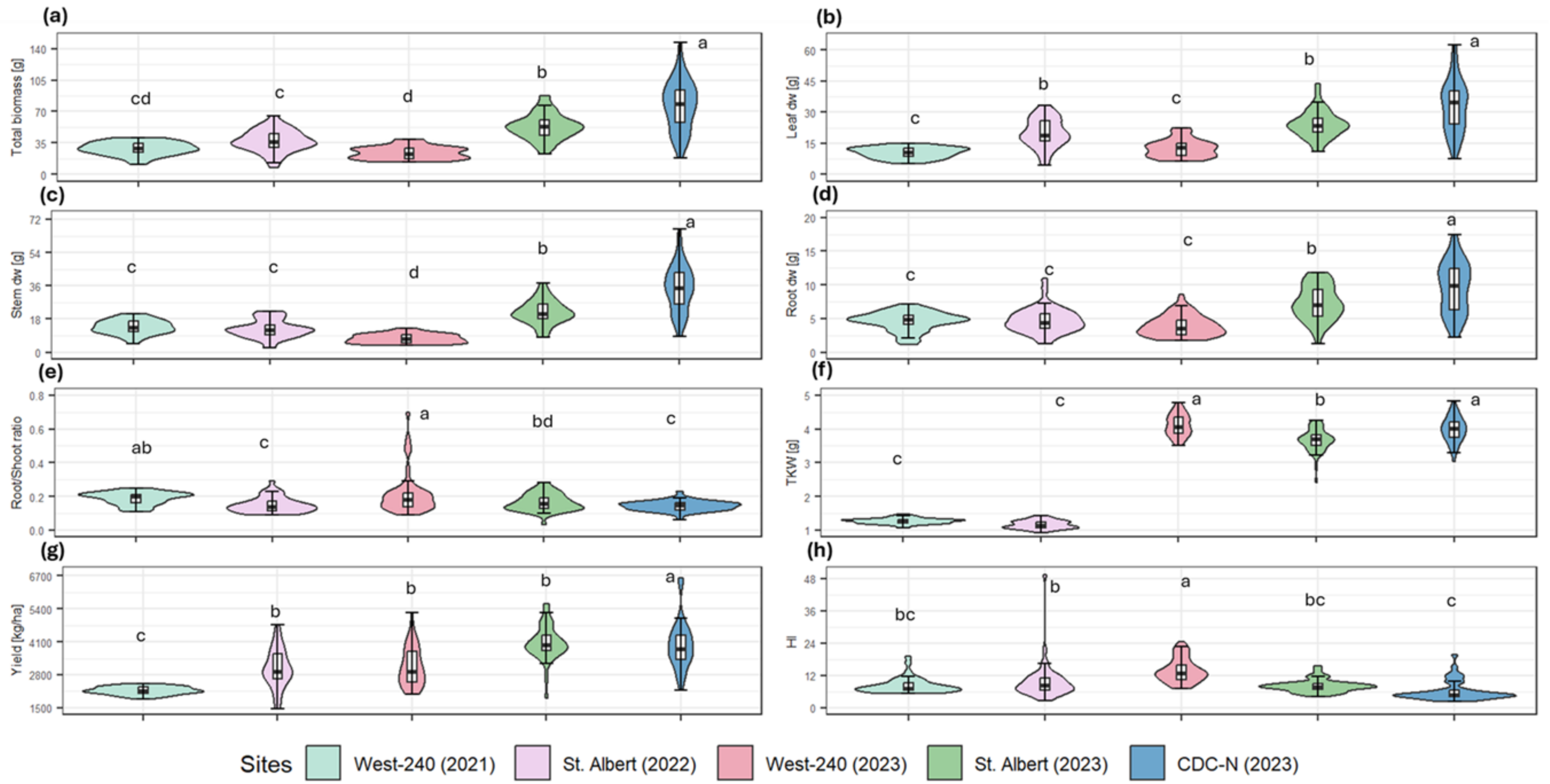

Figure 2. 4 Violin plots combined with boxplots presenting (a) total biomass, (b) leaf biomass (leaf dw), (c) stem biomass (stem dw), (d) root biomass (root dw), (e) root/shoot biomass ratio (R/S), (f) thousand seed weight (TKW), (g) seed yield, and (h) harvest index (HI) of the canola accessions of the (winter canola × spring canola) × rutabaga ((W × S) × R) pedigree group from different site and years. The lowercase letters indicate the similarity or difference based on pairwise comparison with significance threshold of $p = 0.05$.

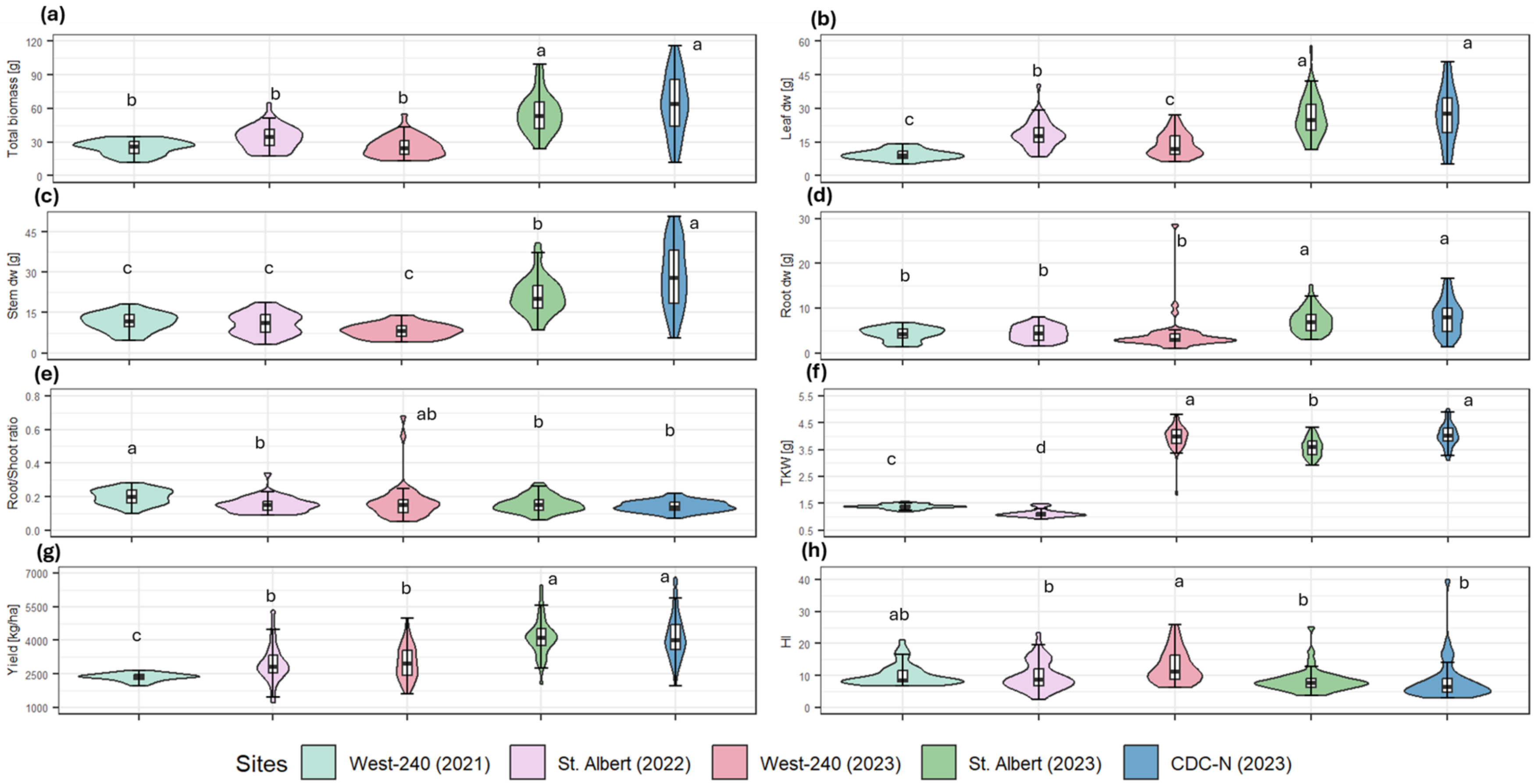

Figure 2. 5 Violin plots combined with boxplots presenting (a) total biomass, (b) leaf biomass (leaf dw), (c) stem biomass (stem dw), (d) root biomass (root dw), (e) root/shoot biomass ratio (R/S), (f) thousand seed weight (TKW), (g) seed yield, and (h) harvest index (HI) of the canola accessions of the S × Oleracea- spring canola ×*Brassica oleracea* pedigree group from different site and years. The lowercase letters indicate the similarity or difference based on pairwise comparison with significance threshold of $p = 0.05$.

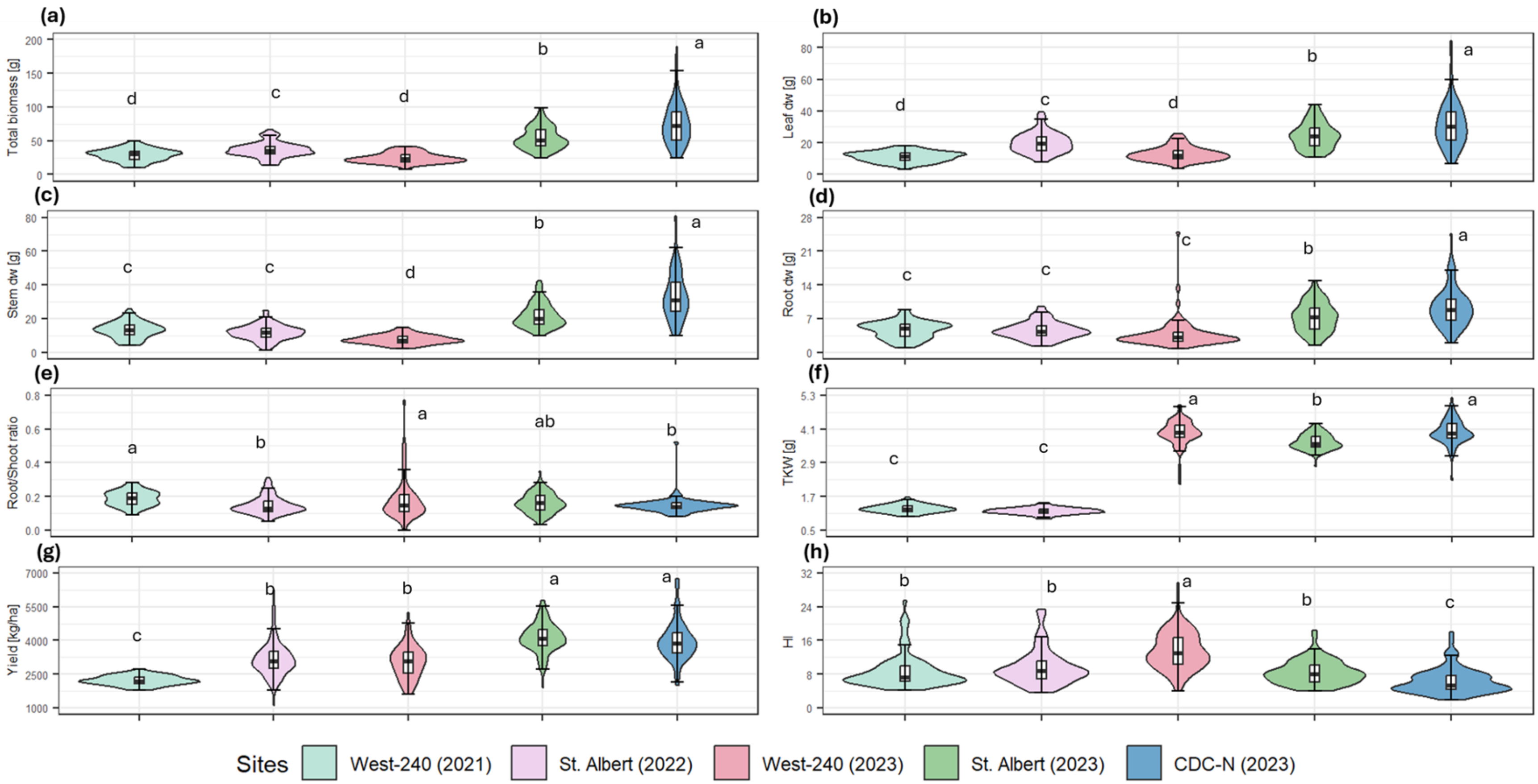

Figure 2. 6 Violin plots combined with boxplots presenting (a) total biomass, (b) leaf biomass (leaf dw), (c) stem biomass (stem dw), (d) root biomass (root dw), (e) root/shoot biomass ratio (R/S), (f) thousand seed weight (TKW), (g) seed yield, and (h) harvest index (HI) of the canola accessions of the S × R- spring canola × rutabaga pedigree group from different site and years. The lowercase letters indicate the similarity or difference based on pairwise comparison with significance threshold of *p* = 0.05.

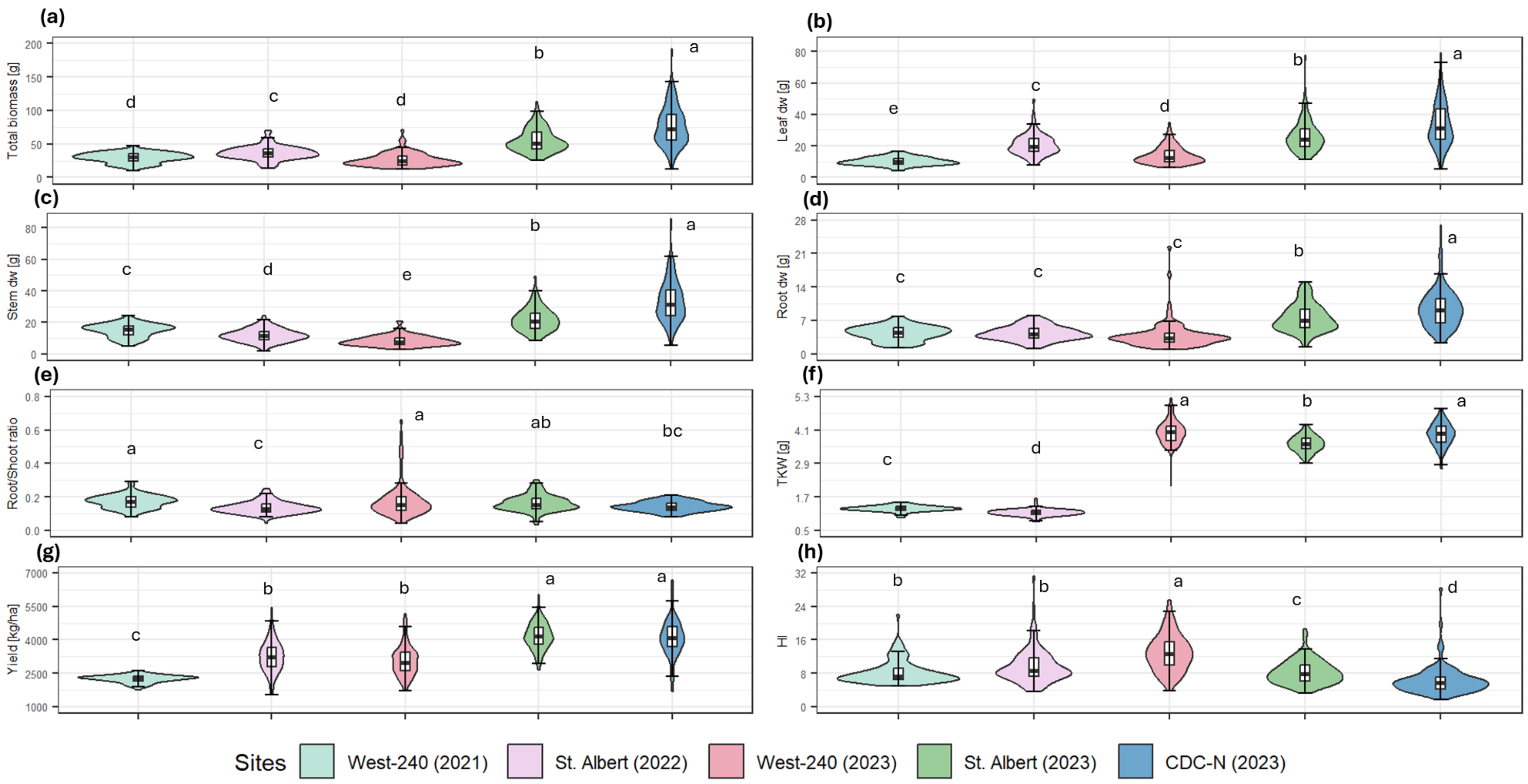

Figure 2. 7 Violin plots combined with boxplots presenting (a) total biomass, (b) leaf biomass (leaf dw), (c) stem biomass (stem dw), (d) root biomass (root dw), (e) root/shoot biomass ratio (R/S), (f) thousand seed weight (TKW), (g) seed yield, and (h) harvest index (HI) of the canola accessions of the S × R- spring canola × rutabaga pedigree group from different site and years. The lowercase letters indicate the similarity or difference based on pairwise comparison with significance threshold of $p = 0.05$.

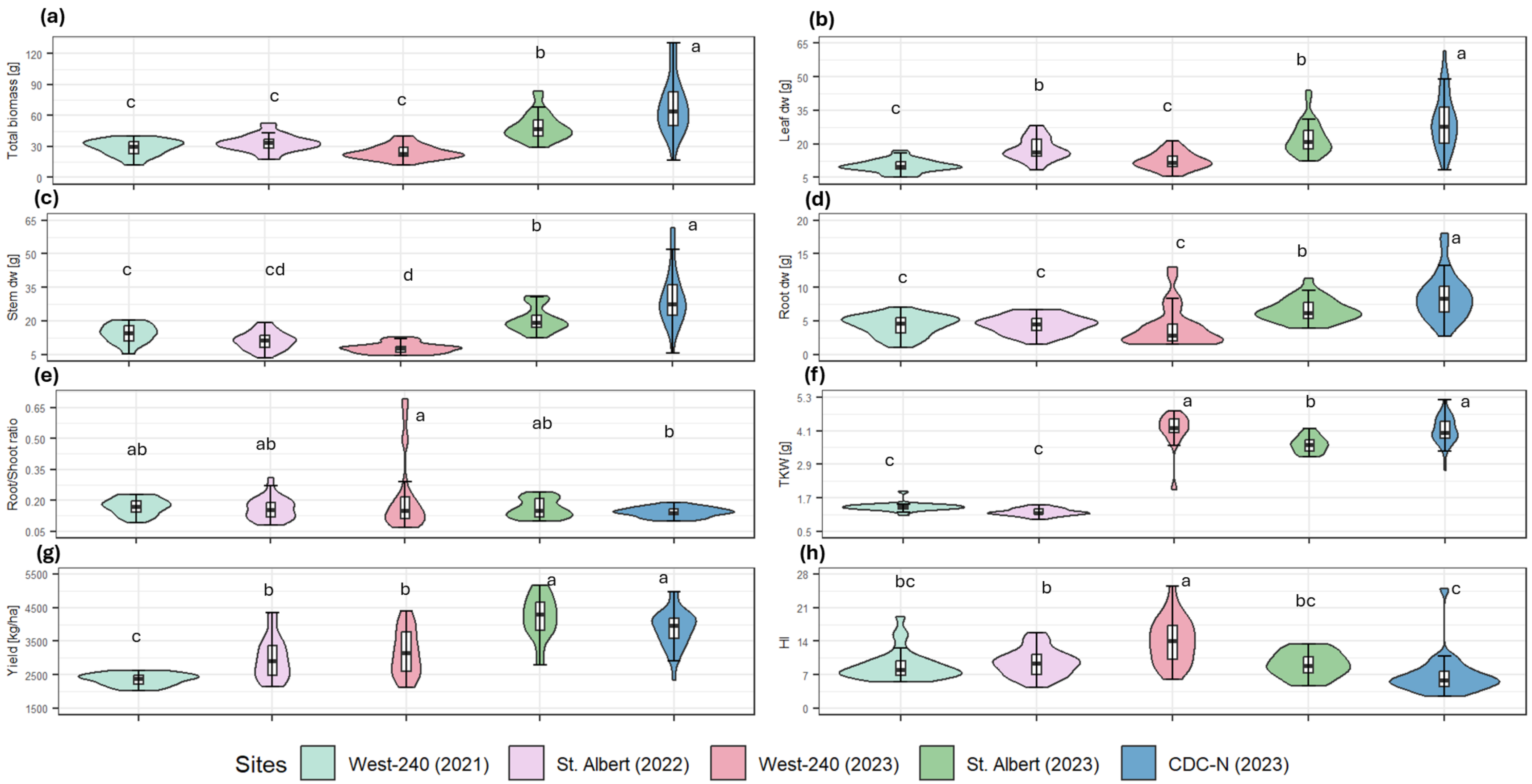

Figure 2. 8 Violin plots combined with boxplots presenting (a) total biomass, (b) leaf biomass (leaf dw), (c) stem biomass (stem dw), (d) root biomass (root dw), (e) root/shoot biomass ratio (R/S), (f) thousand seed weight (TKW), (g) seed yield, and (h) harvest index (HI) of the canola accessions of the S × Rapa- spring canola × *Brassica rapa* pedigree group from different site and years. The lowercase letters indicate the similarity or difference based on pairwise comparison with significance threshold of $p = 0.05$.

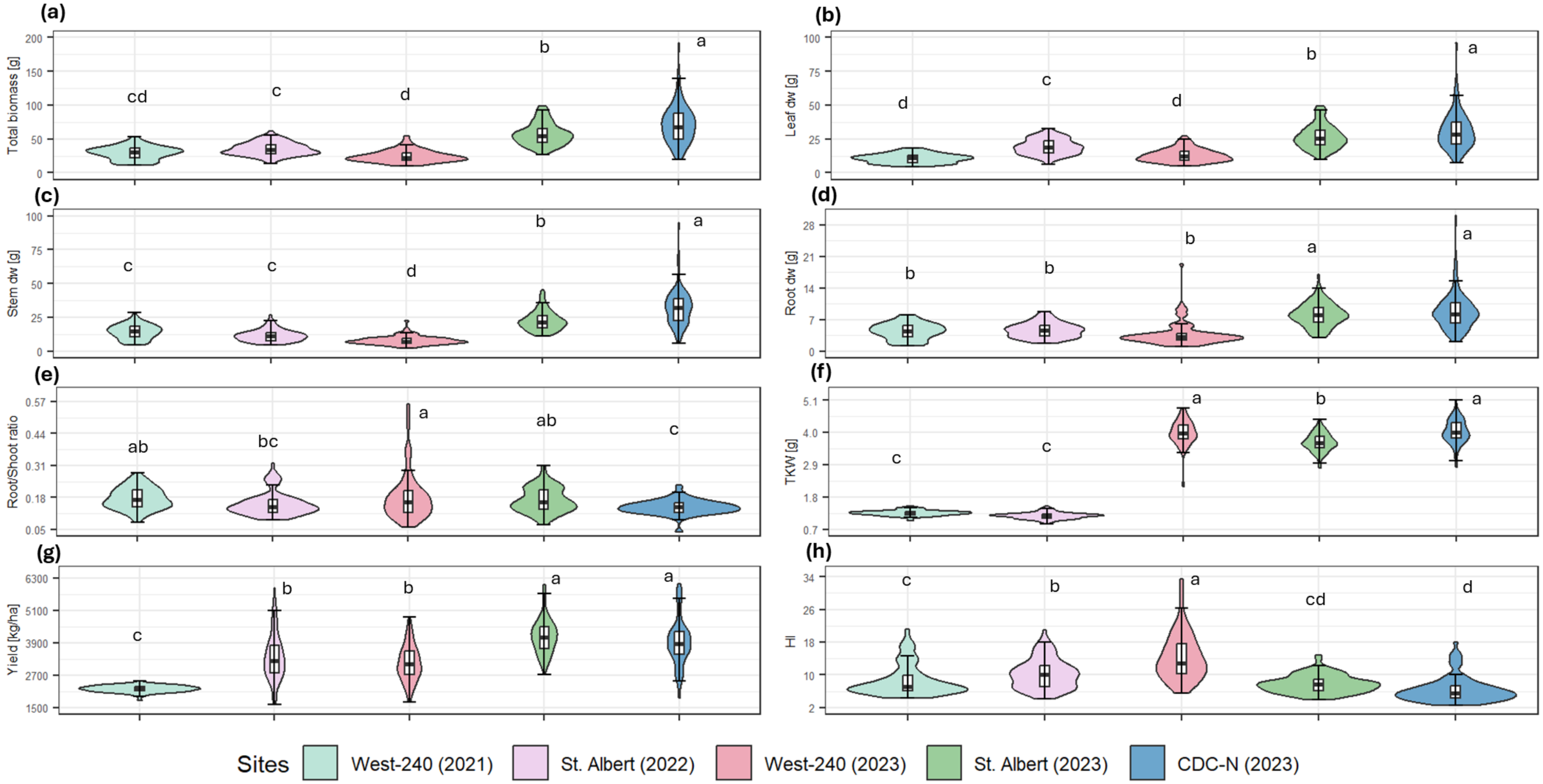

Figure 2. 9 Violin plots combined with boxplots presenting (a) total biomass, (b) leaf biomass (leaf dw), (c) stem biomass (stem dw), (d) root biomass (root dw), (e) root/shoot biomass ratio (R/S), (f) thousand seed weight (TKW), (g) seed yield, and (h) harvest index (HI) of the canola accessions of the W ×S- winter canola × spring canola pedigree group from different site and years. The lowercase letters indicate the similarity or difference based on pairwise comparison with significance threshold of *p* = 0.05.

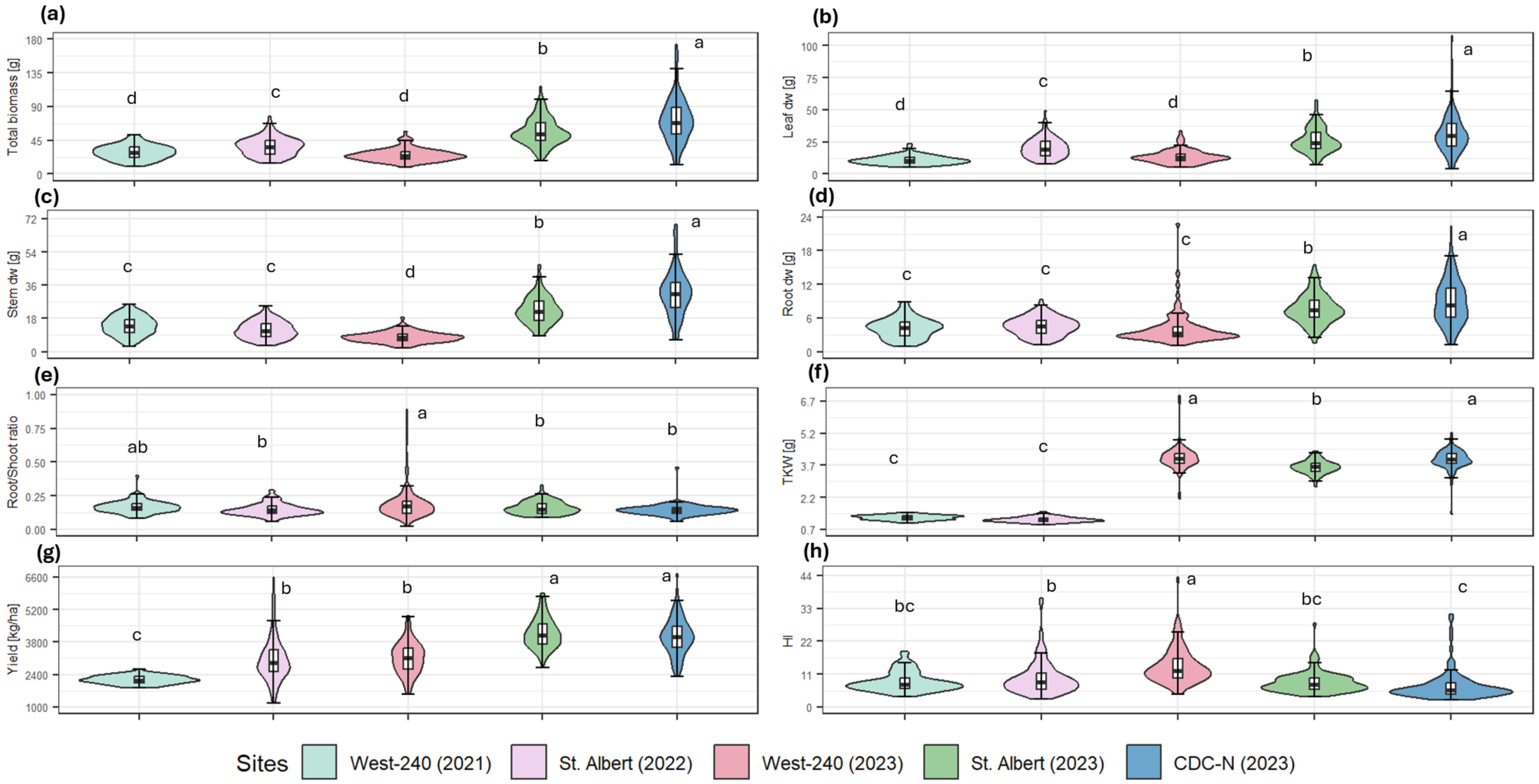

Figure 2. 10 Violin plots combined with boxplots presenting (a) total biomass, (b) leaf biomass (leaf dw), (c) stem biomass (stem dw), (d) root biomass (root dw), (e) root/shoot biomass ratio (R/S), (f) thousand seed weight (TKW), (g) seed yield, and (h) harvest index (HI) of the canola accessions of the gene bank group from different site and years. The lowercase letters indicate the similarity or difference based on pairwise comparison with significance threshold of $p = 0.05$.

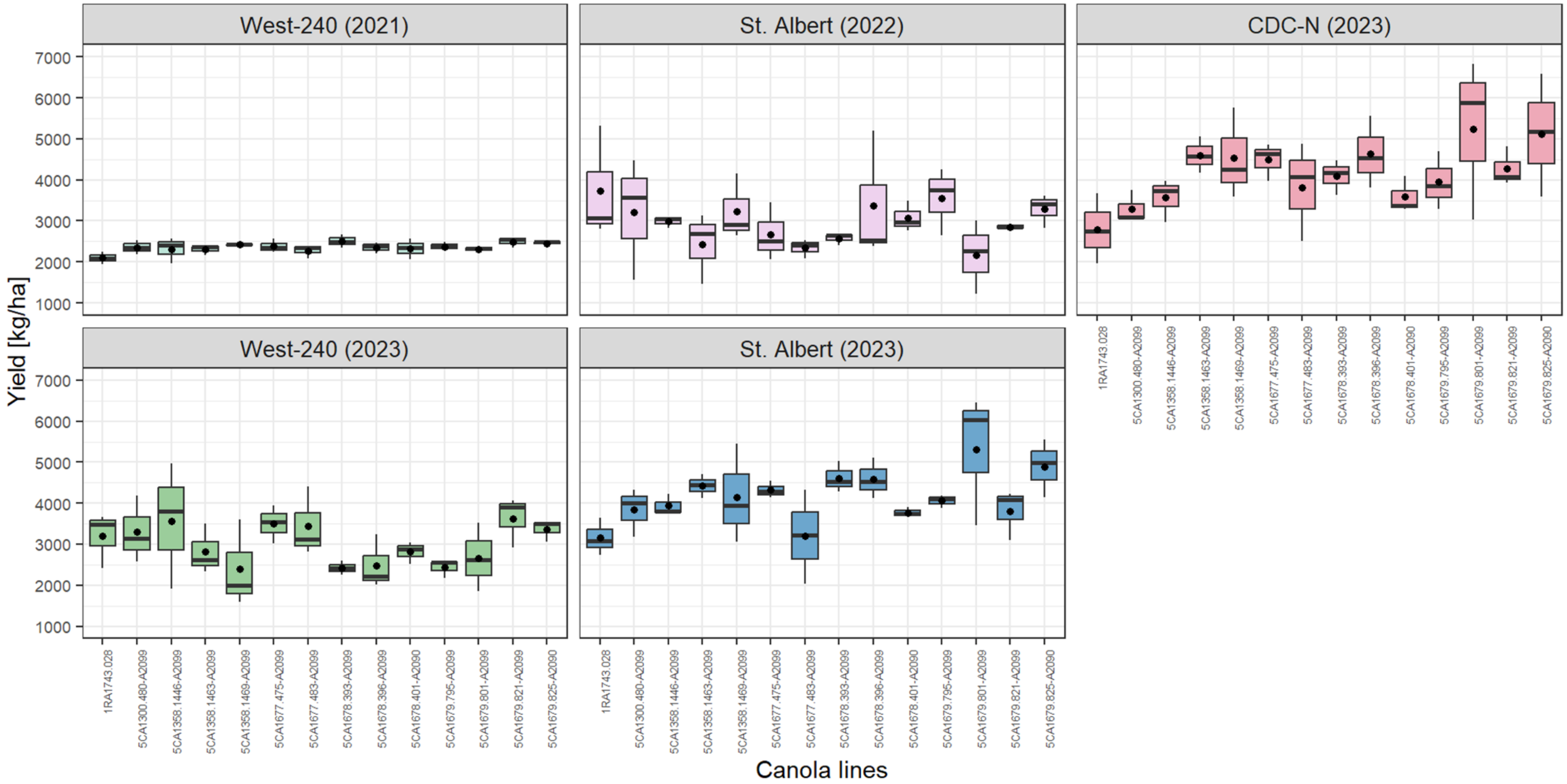

Figure 2. 11 Boxplots presenting the interaction of canola accessions of S × Oleracea- spring canola × *Brassica oleracea* pedigree group with five site years for seed yield. Points in the box indicate the mean and the horizontal bars indicate the median value.

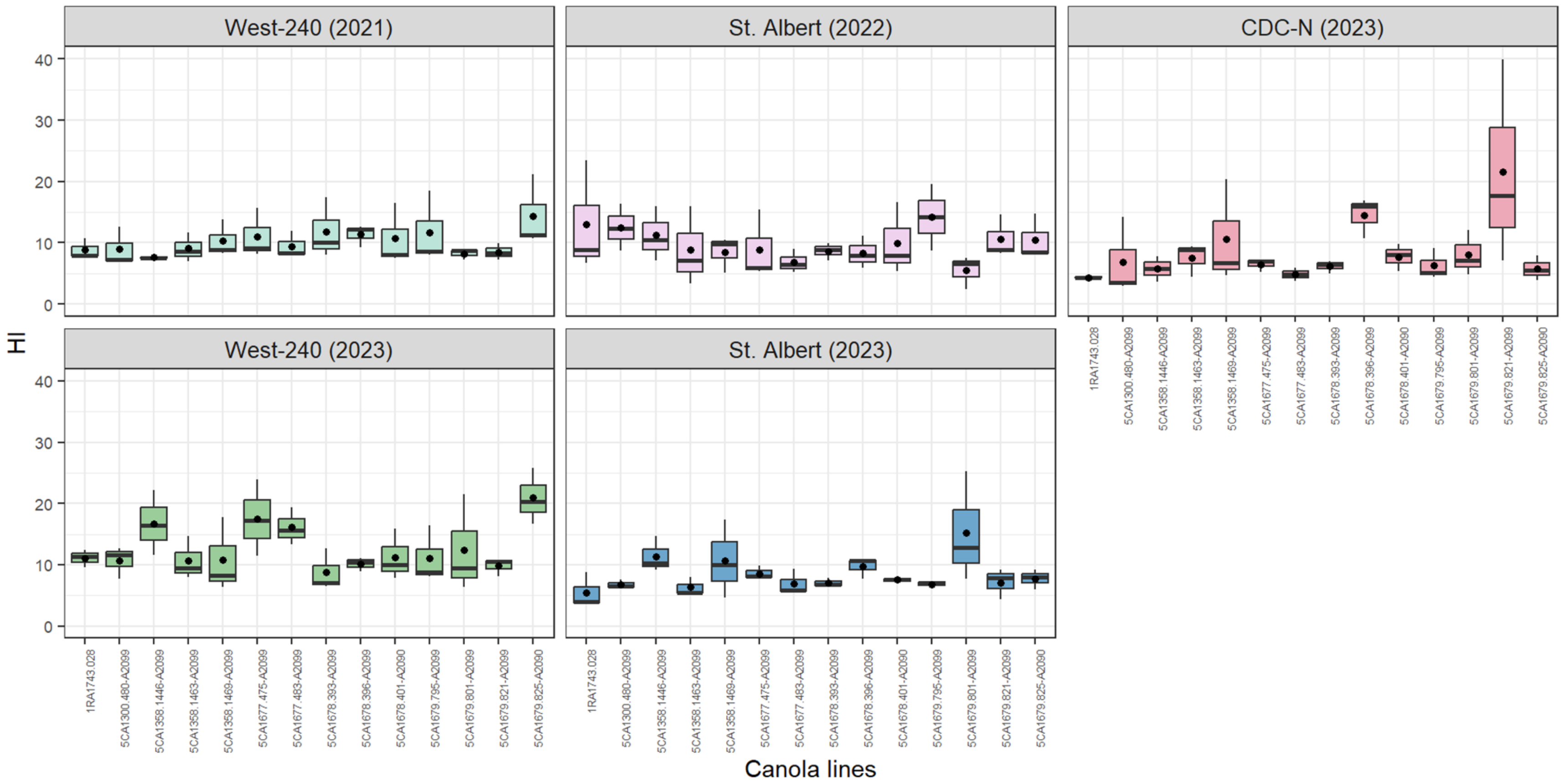

Figure 2. 12 Boxplots presenting the interaction of canola accessions of S × Oleracea- spring canola × *Brassica oleracea* pedigree group with five site years for harvest index (HI). Points in the box indicate the mean and the horizontal bars indicate the median value.

2.3.5. Principal component analysis for agronomic parameters

The PC1 and PC2 together explained more than 75% of the variations in West-240 (2021), which was the highest, while these two PCs explained 64.7%, 56.1%, 60.4% and 62.9% variations for St. Albert (2022), West-240 (2023), St. Albert (2023) and CDC-North (2023), respectively (Fig. 2.13). PCA results will be discussed according to site: At West-240 (2021), the variables: total biomass and leaf, stem and root biomass clustered together, and the cos2 values for these variables were close to 1 which indicates that these variables are positively correlated. The lines for these variables were also close to the perimeter of the circles which indicates that these variables provided greater discrimination among the canola accessions implying that resources, such as photosynthetic assimilates produced in the source (leaves), are effectively partitioned and distributed to other plant parts like sinks (stems, and roots) (Figure 2.13, a). The vector for TKW and seed yield are in the same direction, indicating that these two traits are also positively correlated; this implies that canola accessions with heavier seeds tend to produce higher yields. However, these variables are positioned at almost 90^{o} angle to the biomass variables indicating lack of strong correlation between them. Harvest index is positioned at almost 180^{o} to the biomass variables implying a negative correlation between them as is expected; this also indicates that more efficient partitioning of resources occurred toward grain production rather than vegetative biomass production. R/S ratio had a low cos2 value indicating that this variable provided much less discrimination among canola accessions compared to other variables.

At St. Albert (2022), the biomass variables (total biomass, leaf, stem) are clustered in the same direction indicating that they were positively correlated, and HI was negatively correlated with them (Figure 2.13, b). Seed yield and TKW did not show any relationship with the biomass traits and did not provide much discrimination among canola accessions; this is also evident from very low cos2 values these traits. The PCA biplots from West-240 (2023) and St. Albert (2023) showed a similar pattern where a positive correlation between the biomass variables was evident; the LAI, yield, and TKW had low cos2 values (Figure 2.13, c, d). The PCA from CDC-North (2023) showed that all the biomass variables clustered together as seen in other four sites. R/S ratio, LAI, and seed yield were positioned in different directions to the biomass variables but clustered with each other showing a positive relationship. TKW did not provide much discrimination among the accessions (Figure 2.13, d). The environment plays a significant role on the performance of the canola accessions for all source-sink parameters, and this results in variations in correlations from site to site. Despite this variability, the analyses effectively explained the fundamental concepts of the source-sink relationship.

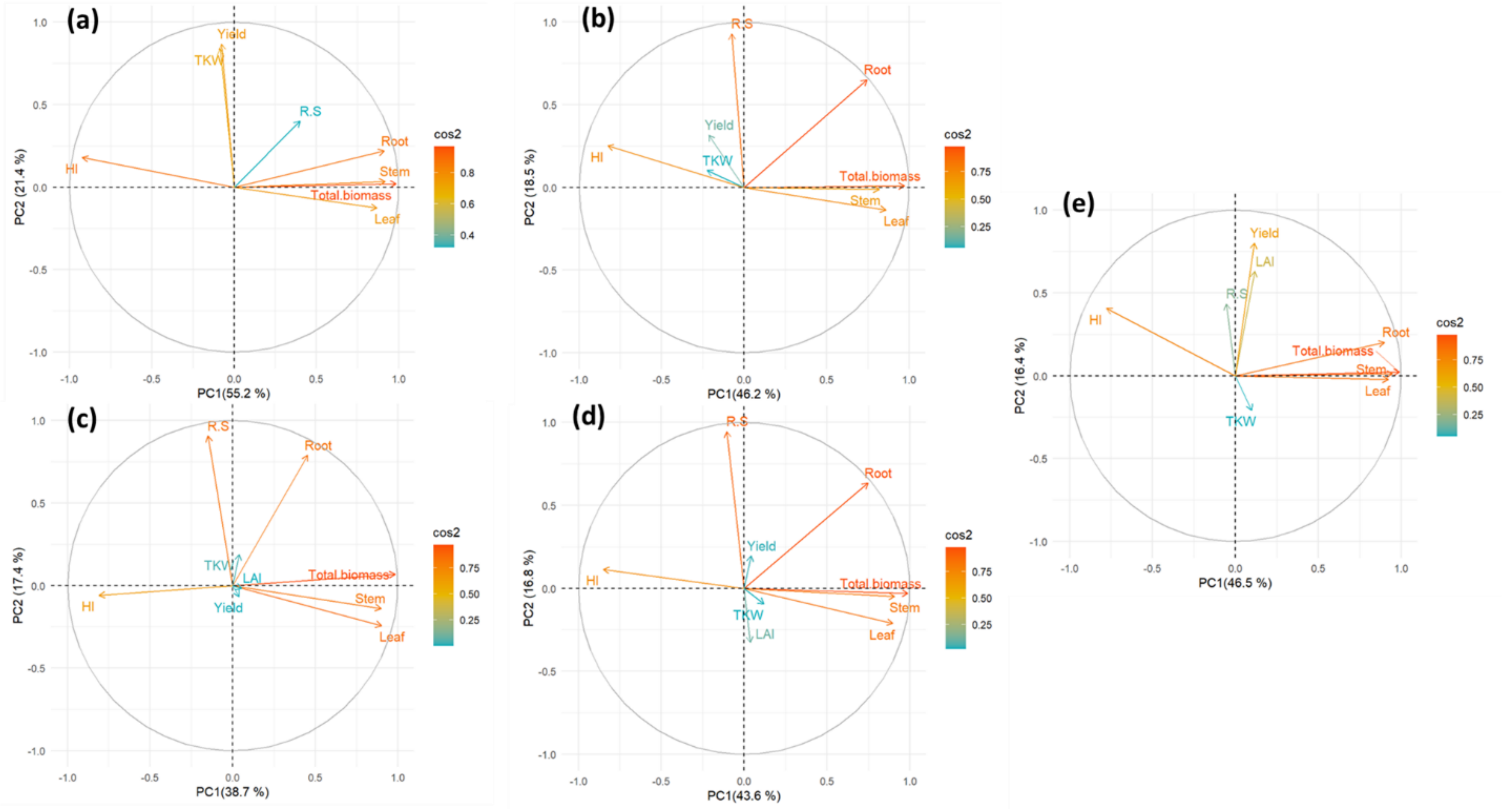

Figure 2. 13 Principal component analysis (PCA) graphs of the 168 canola accessions and two checks from five site years (a) West-240 (2021), (b) St. Albert (2022), (c) West-240 (2023), (d) St. Albert (2023), (e) CDC-N (2023) for resource partitioning variables total biomass, leaf biomass (leaf), stem biomass (stem), root biomass (root), root/shoot ratio (R/S), seed yield, thousand seed weight (TKW), harvest index (HI), and leaf area index (LAI). Cos2 represents the extent of discrimination provided by the variables among the canola accessions.

2.3.6. Identification of canola accessions that outperformed the checks.

The ANOVA (Table 2.1) showed existence of significant variation within the canola accessions mainly for seed yield and thousand seed weight, and the performance of canola accessions was not consistent over the trials. Canola accessions that significantly outperformed the checks for R/S ratio and at least common at two site years was: A01-104NA at West-240 (2023) and CDC-N (2023). Canola accessions that significantly outperformed both checks for seed yield and was at least common at two site years was: 1CA2383.033-A2070 at West-240 (2021 & 2023), and CDC-N (2023)**;** A03-21659NI at St. Albert (2023),and CDC-N (2023); 1IA1082.123-A2090 at West-240 (2021 & 2023); 1RA1488.034 at West-240 (2021), and St. Albert (2022) (see Table 2.2 for details). In terms of TKW, canola accessions that significantly outperformed both checks in at least in two site years were: Crusher at West-240 (2021), and CDC-N (2023); Reston at West-240 (2021), and St. Albert (2023); Turret at West-240 (2021), and St. Albert (2023); 1IA1078.145-A2090 at West-240 (2021 & 2023); IRA1638.084 at West-240 (2021), and CDC-N (2023); IRA1638.085 at West-240 (2021), and St. Albert (2023); IRA1082.103-A2069 at West-240 (2021), and St. Albert (2023); ICA1609.060-A2069 at West-240 (2021), and CDC-N (2023); IRA1003.304 at West-240 (2021), and St. Albert (2023); (Table 2.2). None of the canola accessions outperformed the checks for seed TKW in 2022 (Table 2.2).

Table 2. 2 Canola accessions that significantly outperformed the checks [* significantly higher than check 1 (45H33); **significantly higher than check 2 (L255PC); no star: significantly higher than both checks. [(winter canola × spring canola) × rutabaga ((W × S) × R), spring canola × *Brassica oleracea* (S × Oleracea), spring canola × rutabaga (S × R), spring canola × *Brassica rapa* (S × Rapa) , spring canola × spring canola (S × S), and winter canola × spring canola (W × S) crosses, as well as accessions collected from The Plant Gene Resources of Canada, Saskatoon (Gene bank)]

Canola pedigrees	Sites	Root Dwt	R/S ratio	TKW	Yield
S × Oleracea	West-240 (2021)	None	None	1RA1743.028, 5CA1358.1469-A2099, 5CA1358.1463-A2099, 5CA1677.483-A2099, 5CA1679.795-A2099, 5CA1358.1446-A2099, 5CA1679.825-A2090, 5CA1678.396-A2099, 5CA1300.480-A2099, 5CA1678.393-A2099, 5CA1677.475-A2099, 5CA1679.801-A2099, 5CA1678.401-A2090, 5CA1679.821-A2099	None
	St. Albert (2022)	None	None	5CA1677.475-A2099*, 5CA1677.483-A2099*, 5CA1358.1446-A2099, 5CA1678.393-A2099	None
	CDC-N (2023)	None	None	5CA1679.825-A2090*, 5CA1358.1446-A2099*	None
S × R	West-240 (2021)	None	None	None	1CA2343.052-A2070**, 1CA2354.144-A2070**, 1CA2342.074-A2070, 1CA2506.40-A2040, 1CA2335.080-A2070, 1CA2335.095-A2070, 1CA2383.038-A2070, 1CA2335.069-A2070, 1CA2335.069-A2070, 1CA2354.097-A2070, 1CA2336.142-A2050, 1CA2383.033-A2070, 1CA2354.063-A2070, 1CA2337.046-A2070, 1CA2336.120-A2070, 1CA2337.048-A2070
	St. Albert (2022)	None	None	None	1CA2336.122-A2070*
	West-240 (2023)	None	None	None	1CA2383.033-A2070**, 1CA2342.091-A2050
	St. Albert (2023)	None	None	None	1CA2383.038-A2070*
	CDC-N (2023)	None	None	None	1CA2383.033-A2070*
S × S	West-240 (2021)	None	None	None	A03-22307NA**, A07-26NR**, A05-10NI**, Altex-1**, A04-75NA**, A04-72NA**, Andor**, A03-22808NA**, Quantun, A03-21449NI, A03-22762NA, Hi-Q, Peace, Q2, Conquest, UA BountyGold, A03-22758NA, SILEX, A05-22NA, A01-104NA, Alto, A04-74NA, 1CA0110.004, A06-20NA, A07-28NA, A06-19NA, A04-73NA, A03-22620NA
	St. Albert (2022)	None	None	None	Peace*
	West-240 (2023)	None	A01-104NA*, A03-21449NI*	None	A05-6NI**
	St. Albert (2023)	None	A05-4NI**	None	A03-21659NI*

	CDC-N (2023)	None	A01-104NA**	None	A03-21659NI*
W × S	West-240 (2021)	None	None	1IA1082.121-A2090**, 1RA1584.140**, 1IA1078.137-A2080**, 1IA1078.145-A2090**, 1IA1082.118-A2090**, 1CA1609.061-A2069**, 1IA0944.317-A2099**, 1IA1082.123-A2090**, 1RA1638.084**, 1RA1484.038**, 1IA1078.128-A2080**, 1RA1638.085**, 1CA1616.198-A2069**, 1CA0591.361**, 1IA1082.103-A2080**, 1CA1618.134-A2069**, 1CA1609.060-A2069, A07-35NI, 1RA1498.014, 1IA1078.155-A2090, 1RA1488.034, 1RA1498.016, 1RA1488.014, 1RA1638.100, 1RA1003.304, 1RA1143.141, 1RA1638.102, 1IA0944.312-A4090, UA AlfaGold	1IA1082.123-A2090**, 1IA1082.121-A2090**, 1RA1143.141**, 1CA1609.061-A2069**, 1RA1638.084**, 1IA1082.103-A2080**, 1RA1484.038**, 1CA0591.361**, 1RA1488.034**, 1IA1078.137-A2080**, 1RA1584.140**, 1IA1078.145-A2090**, 1IA1078.128-A2080**, 1RA1498.016**, 1RA1638.100, 1CA1616.210-A2069, 1IA0944.317-A2099, 1RA1488.014, 1RA1003.304, 1RA1638.102, 1RA1498.014, UA AlfaGold, 1IA0944.312-A4090
	St. Albert (2022)	None	1CA0591.361, 1RA1638.102	None	1RA1638.100*, 1RA1488.034*
	West-240 (2023)	1CA0591.361*, 1RA1638.102	None	1IA1078.145-A2090*	1IA1082.123-A2090**
	St. Albert (2023)	1RA1488.014**, 1IA0944.312-A4090	None	1IA1082.103-A2080*, 1RA1003.304*, 1RA1638.085*, 1CA1616.210-A2069*	1CA1618.134-A2069*
	CDC-N (2023)	1RA1638.102	1IA1082.118-A2090**, 1RA1143.141**	1CA1609.060-A2069*, 1RA1638.084*	None
Gene bank	West-240 (2021)	None	None	Pivot**, Next 700**, Crusher**, Zephyr**, Reston**, Golden (non-CQ) **, OAC Triton**, Nugget**, Profit**, Stallion (TR tolerant), OAC Springfield, Regent, Option 501, Senator, AC Excel, Apollo (LL), Oro, Vanguard, Tribute, Tower, AC Tristar, Tanka, Next 500, Target, Westar, OAC Summit, Prominent, Turret	Profit**, Reston**, Nugget**, Golden (non-CQ) **, Apollo (LL)**, AC Tristar**, Option 501**, AC Excel, Tower, Regent, Turret, Tribute, Prominent, OAC Summit, Westar, Vanguard, Next 500, Tanka, Target
	St. Albert (2022)	None	None	None	Hylite 201 (Apetalous)*, OAC Springfield
	West-240 (2023)	None	None	AC Tristar, OAC Summit	None
	St. Albert (2023)	None	None	Turret*, Reston*	None

2.4 Discussion

Source-sink relationships and resource partitioning are reported in various studies over the last several years and demonstrate the interdependence of source-sink organs and the final output in crops performance (Miglani et al., 2021; Fernie et al., 2020; Evans and Clarke, 2019; Biswas et al., 2019; Zuo et al., 2019; Smith et al., 2018; Zhang & Flottmann,2018; Chang et al., 2017; Arvin et al., 2014; Papantoniou et al., 2013; Bennett et al., 2011; Ruan et al., 2010; Yasari et al., 2009; Jeng et al., 2006; Egli & Bruening, 2003). Understanding these relationships elucidates the underlying mechanisms driving the outcomes of this research. The differential allocation of biomass to various plant organs under diverse environmental conditions provides clear evidence of resource partitioning, showcasing how plants optimize their growth and reproductive success by efficiently distributing resources between source organs (leaves and stems) and sink organs (roots and seeds).

Our results indicates that weather conditions tied to year and sites exert a profound influence on canola growth and productivity, as evidenced by the fluctuations of the variables observed during the study period at different site years. In the present study, a significantly lower dry biomass of leaf, stem, and root was found in West-240 (2021) site indicating that fewer resources in the soil were available for plants to uptake with reduced yield due to the drought condition and as a result, yield became less at this site. This result is in agreement with recent reports on source-sink relationship and assimilate partitioning that suggests that plant growth is significantly influenced by environmental factors like temperature, moisture, and nutrient availability (Fatichi et. al., 2014). The dry year (2021) significantly impacted canola yield in our experiment but also Canada wide with a yield reduction of. 35.4% (Canola Council of Canada, 2021). A reason for this yield decrease according to Araus et al. (2002) is that canola is susceptible to water deficit conditions, and this disrupt carbon assimilation and resource allocation. Water stress and high temperature also reduce seed numbers in canola impacting crop yield (Hammac et al., 2017). Cooler temperatures may delay growth stages affecting the timing of flowering and maturity of the crops. Furthermore, fluctuations in vapor pressure deficit (VPD) further underscore the importance of moisture availability in shaping canola performance. High VPD levels, particularly during drought periods, can exacerbate water stress, leading to reduced photosynthetic activity and yield losses (Flexas et al., 2002). Between 1990 and 2010, changes in management and production practices in Canadian prairies led to not only increased canola yields but also optimized resource utilization, contributing to enhanced environmental performance per tonne of canola produced (MacWilliam et al., 2016). On the other hand, at CDC-North 2023 site the weather was optimum, resulting in better resource allocation in the source and sink organs leading to greater biomass production and allocation resulting in increased yield. The higher biomass and yield observed in some of the pedigree groups, particularly in CDC-North (2023), can be attributed to efficient resource partitioning strategies, favoring allocation to sink organs such as the seeds. This aligns with the principle of source-sink dynamics, where optimal allocation of assimilates to developing seeds enhances yield potential (Shi et al., 2013). Faraji (2014) also reported that optimum soil conditions and resource availability is essential for healthy source-sink relationships and efficient resource partitioning, thus, for maximizing crop productivity in canola. Weymann et al. (2015) carried out a field trial with winter oilseed rape in 34 environments and reported that 40% of the yield variability was due to the weather conditions that influence assimilate availability and source-sink interactions. Our research results support this, showing varying yield responses across different sites. This suggests a source limitation, especially during the late reproductive phase, with compensatory effects observed between seed number and seed weight. Variations in biomass allocation (total biomass, leaf, stem, root, R/S ratio) and yield parameters were notable across different site years in this study. LAI at CDC-North (2023) was significantly higher than the other sites; this LAI showed a positive correlation with seed yield. Li et al. (2019) also showed that LAI is closely associated with crop growth, development, and yield potential. Leaf anatomical features, including stomatal density and leaf size, play crucial roles in influencing LAI and photosynthetic efficiency through their impact on light interception, gas exchange, and water relations. Canola cultivars with higher LAI and more radiation use efficiency increase photosynthetic activity and assimilate production contributing to enhanced resource allocation to developing sinks, such as seeds, resulting in greater biomass accumulation and ultimately higher yield (Biabani et al., 2021).

R/S ratio, an indicator of biomass allocation between aboveground and belowground organs, remained relatively stable across the site-years. This suggests a consistent allocation strategy aimed at balancing resource uptake and utilization, essential for maintaining plant growth and resilience (Lambers et al., 2008). Our study shows a balanced R/S ratio ensures that the plant can sustain its growth and maintain physiological functions even under stress conditions (Thiagarajan et al., 2018). For instance, a robust root system can support the plant during water-deficit conditions by accessing deeper soil moisture, while a healthy shoot system can maximize photosynthetic output during favorable conditions. However, the lower representation of the R/S ratio in the PCA biplot indicates it exerted lesser influence on overall variability in the population as compared to other variables, highlighting the dominance of aboveground biomass in driving yield outcomes. Principal Component Analysis (PCA) further elucidated the relationships between different variables, providing insights into their collective impact on canola performance. The clustering of biomass variables together with LAI, yield, and TKW indicates their strong positive correlation, implying that these may be the most important variables influencing yield potential. Conversely, the negative correlation of harvest index with biomass variables suggests trade-offs between vegetative growth and reproductive efficiency, reflecting the allocation of assimilates to seed production (Sierra-Gonzalez et al., 2021). These findings underscore the importance of balancing source-sink dynamics to optimize yield and resource use efficiency in canola cultivation. Canola accessions showing significantly higheryield and TKW can be attributed to optimized resource partitioning and efficient source-sink relationships. Significantly higher yield and TKW in the specific canola accessions that were observed in this study likely resulted from enhanced source activity resulting in increased assimilate production and efficient allocation of resources and directing a larger proportion of assimilates towards the developing sinks, such as the seeds. Additionally, these high-performing canola accessions may demonstrate improved sink strength, facilitating enhanced seed filling and seed development that indicates balanced source-sink dynamics in these canola accessions.

The results obtained from the current study highlight the intricate interplay between environmental factors, genetic attributes, and resource allocation strategies in shaping canola productivity. Source-sink dynamics and resource partitioning provide valuable insights in this study for breeding programs aimed at enhancing canola resilience and productivity in diverse agroecosystems. Canola accessions that significantly outperformed the checks in terms of root-to-shoot ratio (R/S), seed yield, and thousand-kernel weight (TKW) across multiple site years provide strong evidence of effective source-sink dynamics and resource partitioning. The accession A01-104NA, which excelled in R/S ratio at West-240 (2023) and CDC-N (2023), indicates a balanced allocation of biomass between aboveground and belowground organs, essential for sustaining growth and maximizing nutrient uptake and more suitable for dry environment with more root biomass. Canola accessions such as 1CA2383.033-A2070 at West-240 (2021), West-240 (2023), and CDC-N (2023); A03-21659NI at St. Albert (2023) and CDC-N (2023); 1IA1082.123-A2090 at West-240 (2021) and West-240 (2023); and 1RA1488.034 at West-240 (2021) and St. Albert (2022), which consistently outperformed both checks in seed yield across different site years, demonstrate superior sink strength and efficient assimilate distribution to reproductive organs, leading to increased yields. High-performing canola accessions like Crusher at West-240 (2021) and CDC-N (2023); Reston at West-240 (2021) and St. Albert (2023); Turret at West-240 (2021) and St. Albert (2023); 1IA1078.145-A2090 at West-240 (2021) and West-240 (2023); IRA1638.084 at West-240 (2021) and CDC-N (2023); IRA1638.085 at West-240 (2021) and St. Albert (2023); IRA1082.103-A2069 at West-240 (2021) and St. Albert (2023); ICA1609.060-A2069 at West-240 (2021) and CDC-N (2023); and IRA1003.304 at West-240 (2021) and St. Albert (2023) with respect to TKW at multiple sites suggest optimized biomass allocation to seeds, enhancing reproductive efficiency and final grain weight. These results highlight the critical role of genetic traits that favor effective resource partitioning with resultant biomass allocation, enabling canola plants with superior performance to thrive under varying environmental conditions. Future research regarding genomic techniques, and breeding methodologies will help in unraveling the underlying mechanisms of different canola accessions' performance.

Chapter 3 Characterization of stomata and trichomes in different canola accessions under field conditions

3.1. Introduction

The Brassicaceae genus comprises various species utilized for multiple purposes, including oilseed, forage, condiment, and vegetable crops, with *Brassica oleracea*, *B. rapa, B. napus*, and *B. juncea* being the most important ones (Cartea et al., 2011). These crops, such as kale, cabbage, turnip, and mustard, are significant components of diets globally, particularly in China, Japan, India, and European nations, offering versatility in culinary and agricultural applications (Cartea et al., 2011). Brassicaceae family is comprised of a total of 340 genera and 3700 species (Pedras & Yaya, 2010). In Canada, canola is a major oilseed crop and plays a significant role in the country's agricultural industry. In canola like other higher plants leaves are the main organs where assimilates are produced in the process of photosynthesis (Castro-Díez et al., 2000). Green leaves absorb solar energy and influence leaf photosynthesis (Bolhàr-Nordenkampf & Draxler, 1993). Leaves have anatomical structures that participate in the photosynthesis process. Therefore, studying different leaf anatomical structures is crucial to improve crop plants productivity. Furthermore, an understanding of canola leaf morphology, specifically trichomes (hair-like structures) and stomata (small pores) may help in crop management practices such as pest control and water use efficiency. Stomata are small openings on the leaves epidermal layer that regulate gas exchange, facilitating photosynthesis and oxygen release while managing water loss through transpiration. Their strategic positioning and dynamic response to environmental factors optimize both photosynthesis and water conservation, crucial for the plant's adaptation to varying conditions (Buckley, 2005). Stomatal density in canola shows a positive correlation with water use efficiency and this knowledge can be used to develop canola cultivars resistant to water-limited conditions (Raman et al., 2020). In addition to stomatal density, other epidermal features such as trichomes also play a crucial role in plant adaptation to environmental conditions. Trichomes, emerging from the epidermal tissue, manifest as hair-like projections on various plant surfaces, often discernible to the naked eye (Johnson, 1975). Trichomes showcase a diverse array of forms, each serving distinct functions such as ameliorating heat and water loss and providing defense against pests through either chemical secretion or physical barriers (Szymanski et al., 2000). In the pursuit of insect resistance, canola researchers have sought inspiration from other species, incorporating trichomes from diverse sources. For instance, the introduction of the extremely hairy Arabidopsis gene into *B. napus* has shown promise in conferring protection against flea beetles (Gruber et al., 2006). Noteworthy research on two genetically modified *B. napus* lines, boasting enhanced trichome densities, has demonstrated reduced insect feeding and heightened antibiosis resistance against pests like flea beetles and diamondback moths, all without compromising vital agronomic traits such as seed yield (Alahakoon et al., 2016). Despite the pivotal role of stomata and trichomes in canola physiology, they have not been characterized in existing canola accessions. Therefore, this study embarks on a novel dimension, scrutinizing different canola accessions to unravel the anatomical nuances of these vital structures. By shedding light on the intricacies of trichomes and stomata in canola leaves, this research aims to unveil crucial insights into their structures and distribution, paving the way for enhanced crop management strategies and breeding programs.

3.2 Material and Methods

3.2.1 Plant material

Sampling was done at West-240 (2021) on 168 canola accessions and two checks. The canola accessions were derived from seven different pedigree groups including (winter canola × spring canola) × rutabaga ((W × S) × R), spring canola × *Brassica oleracea* (S × Oleracea), spring canola × rutabaga (S × R), spring canola × *Brassica rapa* (S × Rapa) , spring canola × spring canola (S × S), and winter canola × spring canola (W × S) crosses, as well as canola accessions collected from The Plant Gene Resources of Canada, Saskatoon (Gene bank); the checks were L255PC (LibertyLink) and 45H33 (Roundup Ready).

**See appendices supplementary table 2.1 for canola pedigree group abbreviation*

3.2.2. Surface imprint collection

Imprints of the leaves' adaxial and abaxial surfaces were made for 540 plots (168 canola accession, two checks with three replication) at BBCH 50 on the first fully developed leaf. Suzuki's Universal Micro-Printing (SUMP) discs (Sump Laboratory, Tokyo, Japan) with SUMP liquid were used to prepare the slides (Tanaka et al., 2005). The SUMP disc was affixed to glass slides using double-sided tape and labeled. These imprints were visualized using a Zeiss Primo Star light microscope at 10x magnification and hand-counted the stomata, trichomes, and epidermal cells. For each accession, images from three fields of view were captured for each sample. Stomata, trichome and epidermal cells at both adaxial and abaxial surface were counted using the Zen software. Based on this, trichome density, stomatal density, and epidermal cell density were calculated using the following formula:

$$Cell\ density = \frac{Number\ of\ cells}{Area}$$

The stomatal index was calculated using the formula:

$$Stomatal\ index\ (\%) = \frac{Number\ of\ stomata\ per\ unit\ area}{(Number\ of\ stomata\ per\ unit\ area + Number\ of\ epidermal\ cells\ per\ unit\ area)\ \times 100}$$

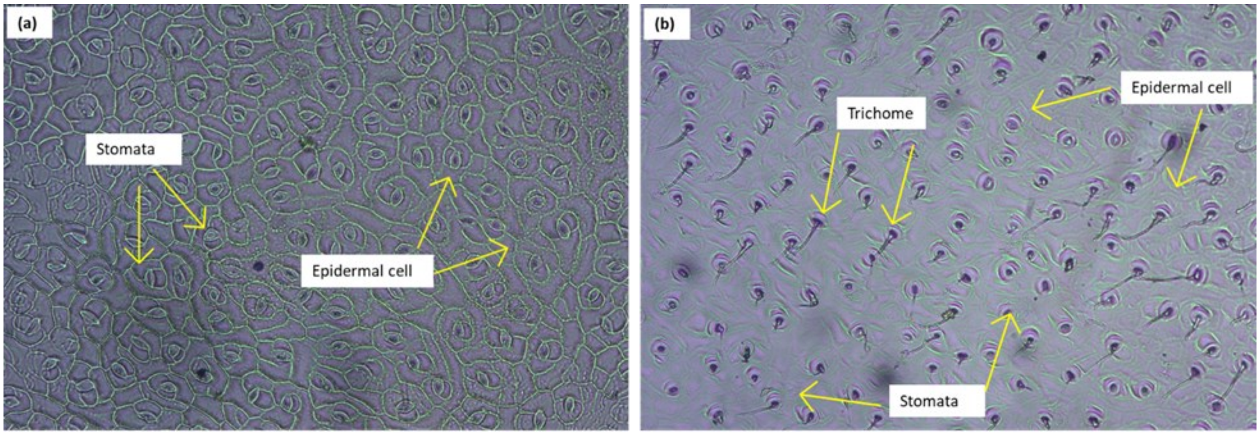

Figure 3. 1 Representative image of a leaflet imprint at $10x$ magnification used to quantify micromorphological traits on the surface showing stomata, epidermal cells, and trichomes. (a) Nugget [gene bank] (b) A04-75NA [S × S]

3.2.2 Statistical analysis

All analyses were conducted using R version 4.1.1 (R Core Team, 2021). An analysis of variance was performed to identify the significant sources of variation. In this study, various canola accessions and pedigree groups served as independent or predictor variables, while morphological parameters were considered response variables. The two-way ANOVA model, implemented with the above function in R (R Core Team, 2021), investigated the individual effects of canola accessions, represented by the following formula:

Morphological variables ~ Accessions + Pedigree groups

Significance levels for different canola accessions for these traits and their interactions were observed at $p<0.001$, $p<0.01$, and $p<0.05$ (Table 3.1). The analysis involved transforming the variables in the dataset using the dplyr package (Wickham et al., 2022), and converting them into factors to facilitate the subsequent analyses. Boxplots were generated with ggplot2 package (Wickham, 2016) to visually compare the morphological variables across different canola pedigree groups. Additionally, the agricolae package (De Mendiburu & Yaseen, 2020) was utilized for conducting Tukey's Honestly Significant Difference (HSD) test for significant differences between the groups. The correlation plot was created using the pairs panels function of the psych package (Revelle, 2022) to visualize pairwise correlations between the variables. The plot included histograms on the diagonal, Pearson correlation coefficients, and ellipses to represent the strength and direction of correlations. Simple linear regression was performed since some variables had higher correlation coefficients using the following formula:

$$y = a + bx$$

where y is the dependent variable, a is the intercept, b is the regression coefficient or slope, and x is the independent variable. The regression plot was generated using ggplot2 (Wickham, 2016), ggpubr (Kassambara, 2020), ggpmisc (Akkerman, 2022), GGally (Schloerke et al., 2021), and tidyr (Wickham et al., 2024) packages. It included scatterplots of the stomatal density against the epidermal cell density across the canola pedigree groups. Additionally, linear regression lines were added to each facet along with equations, R-squared values, and p-values. Emmeans comparisons were conducted to identify the superior canola accessions compared to the controls. Using the lmer function from the lme4 package (Bates et al., 2015), a linear mixed-effects model was fitted to the data, incorporating fixed effects of canola accessions and random intercepts for replication and experimental blocks. Estimated marginal means were computed using the emmeans function from the emmeans package (Lenth, 2021) for pairwise comparisons between the accessions. Adjustment for multiple comparisons was executed with the cld function from the multcomp package (Horthorn et al., 2008).

3.3 Results

3.3.1. Leaf morphological structures

Analysis of variance revealed that significant variation existed among the canola accessions and pedigree groups for adaxial stomatal density (SDAD), abaxial stomatal density (SDAB), adaxial epidermal cell density (EDAD) and abaxial epidermal cell density (EDAB) (Table 3.1). In addition to this, significant variation was found among the canola accessions for adaxial stomatal index (SIAD). No significant variation was found among canola accessions and pedigree groups for abaxial stomatal index (SIAB), adaxial trichome density (TDAD) and abaxial trichome density (TDAB). Boxplots of the canola pedigree groups viz. (W × S) × R: winter canola × spring canola × rutabaga; S × Oleracea: spring canola × *Brassica oleracea*; S × R: spring canola × rutabaga; S × Rapa: spring canola × *Brassica rapa*; S × S: spring canola × spring canola; W × S: winter canola × spring canola; and Gene bank, and the checks L255PC and 45H33 are presented in Figure 3.2. SDAD and EDAD showed a similar pattern across the different canola pedigree groups where the canola pedigree group S × R, showed significantly greater SDAD and EDAD. This indicates that a significantly higher number of stomata and epidermal cell per unit area on the leaf surface was present in this pedigree group. (Figure 3.1, a, c). In the terms of SDAB and EDAB, most canola pedigree groups were similar to the checks, and both variables showed a similar pattern; this indicates that the ratio of epidermal cell and stomatal cells was similar in the leaf of canola accessions (Figure 3.1, b, d).

Table 3. 1 Analysis of variance of the canola accessions and pedigree groups (PG) for adaxial stomatal density (SDAD), abaxial stomatal density (SDAB), adaxial epidermal cell density (EDAD), abaxial epidermal cell density (EDAB), adaxial stomatal index (SIAD), abaxial stomatal index (SIAB), adaxial trichome density (TDAD), and abaxial trichome density (TDAB). ***, **, and * represents significance at 0.001, 0.01 and 0.05 probability levels. ns means non-significant.

Sources of variation	SDAD	SDAB	EDAD	EDAB	SIAD	SIAB	TDAD	TDAB
Accessions	0.001**	0.002**	0.004**	0.003**	0.02*	ns	ns	ns
PG	<0.00***	0.002**	<0.00***	0.002**	ns	ns	ns	ns

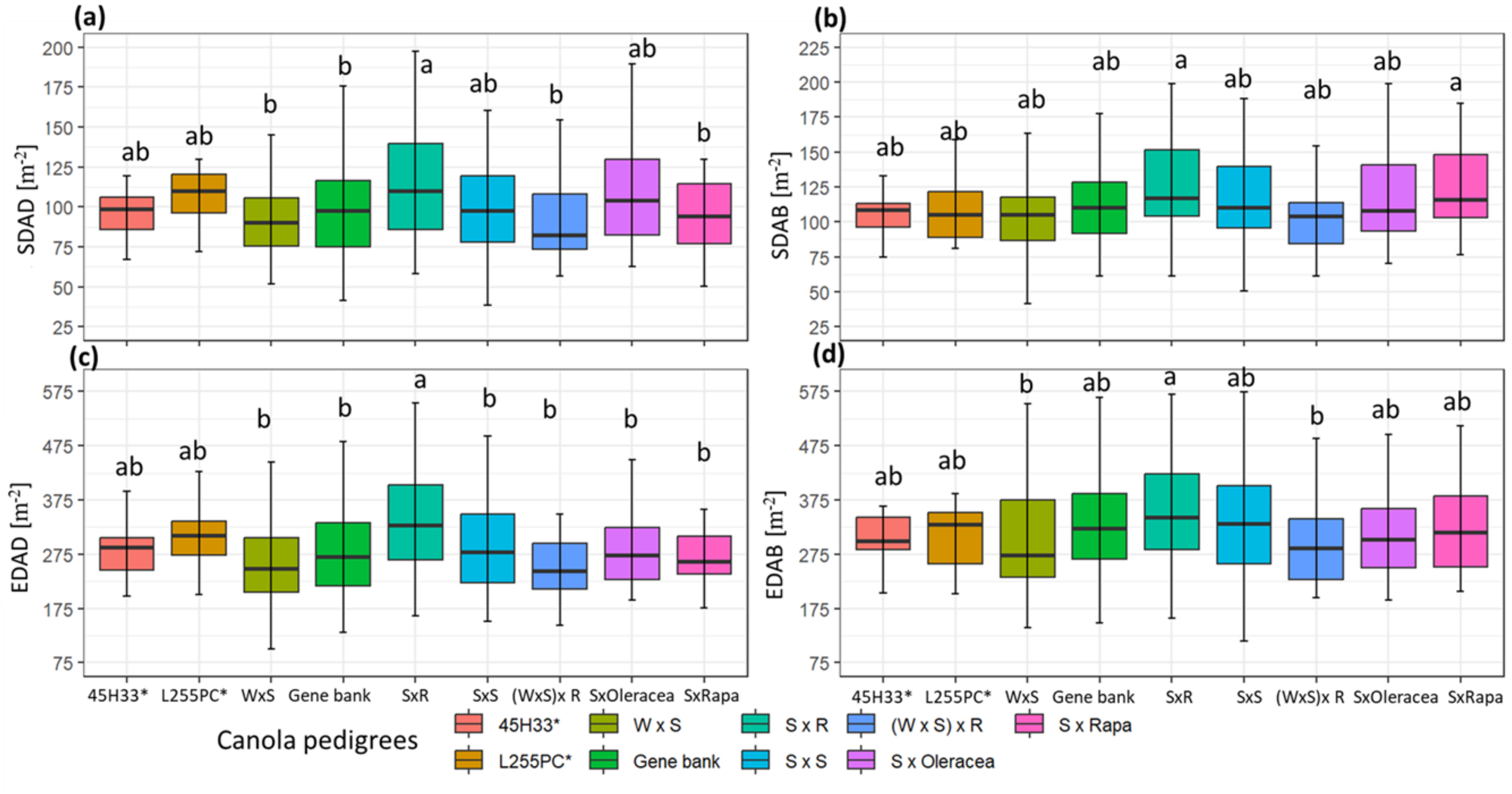

Figure 3. 2 Boxplots for (a) adaxial stomatal density (SDAD), (b) abaxial stomatal density (SDAB), (c) adaxial epidermal cell density (EDAD), (d) abaxial epidermal cell density (EDAB) of the different canola pedigree groups. Box plots with the same letter at the top are not significantly different. Tukey test was performed with a *p* value of 0.05 probability levels. [(winter canola × spring canola) × rutabaga ((W × S) × R), spring canola × *Brassica oleracea* (S × Oleracea), spring canola × rutabaga (S × R), spring canola × *Brassica rapa* (S × Rapa) , spring canola × spring canola (S × S), and winter canola × spring canola (W × S) crosses, as well as accessions collected from The Plant Gene Resources of Canada, Saskatoon (Gene bank)].

3.3.2. Trichome density

Trichome was not present in all 168 canola accessions and 2 checks evaluated. For this trait, a simple analysis was done to indicate the proportion of canola accessions belonging to different canola pedigree groups that have trichomes (Table 3.2). About 19-21% of canola accessions in the pedigree groups, S × S, W × S, gene bank, (W × S) × R, and S × Oleracea groups had trichomes on the adaxial surface; only about 10% of canola accessions in pedigree group, S × Rapa had trichomes on their adaxial surface. For abaxial surface, about 36% of canola accessions in pedigree group, S × Oleracea had trichomes, while only about 10% in pedigree group, (W × S) × R and Gene bank accessions had trichomes.

Table 3. 2 Percentage of adaxial and abaxial trichomes present in different canola pedigree groups.

Canola pedigree groups	Number of accessions	Percent accessions with adaxial trichomes	Percent accessions with abaxial trichomes
(W × S) × R	14	21	14
Gene bank	35	20	14
S × Oleracea	14	21	36
S × R	28	11	25
S × Rapa	10	10	20
S × S	37	19	16
W × S	30	20	17

Trichome present in both checks L255PC*, 45H33*. [(W × S) × R: winter canola × spring canola × rutabaga; S × Oleracea: spring canola × *Brassica oleracea*; S × R: spring canola × rutabaga; S × Rapa: spring canola × *Brassica rapa*; S × S: spring canola × spring canola; W × S: winter canola × spring canola; Gene bank]

3.3.3. Relationship between leaf morphological parameters

SDAD and EDAD showed a strong positive correlation with Pearson correlation coefficient of 0.70 (Figure 3.3). SDAB and EDAB also showed a strong positive correlation with a Pearson correlation coefficient of 0.71 (Figure 3.3). The regression between SDAD and EDAD for all canola pedigree groups showed a significantly higher R square value, where the significantly strongest relationship was found in the check, 45H33 and canola pedigree group, S × S ($R^2 = 0.54$). (Figure 3.4). For SDAB and EDAB, all canola pedigree groups showed a significant linear relationship (Figure 3.5). Significantly higher ($p<0.001$) R-square value (0.64) was found in S × Oleracea. Therefore, canola accessions with high stomatal density implies high epidermal cell density and vice versa. Canola accessions outperforming the checks in terms of anatomical variables are listed in the emmeans comparison table (Table 3.3). Few canola accessions from certain canola pedigree groups outperformed the checks in at least two of the variables and this include the accessions Wester (SDAB, EDAB) of the Gene Bank pedigree group, 5CA1679.821-A2099 (SDAD, SDAB) from S × Oleracea, 1IA2519.005-A2000 (SDAD, SDAB, EDAD, EDAB), 1CA2337. 090.A2050 (SDAD, SDAB, SIAD) and 1CA2354.144-A2070 (SDAD, SDAB, EDAB) from S × R, Alto (SDAB, EDAB) from S × S , and 1RA1638.102 (SDAB, EDAB) and 1CA1609.060-A2069 (SDAB, EDAB)from W × S (Table 3.3).

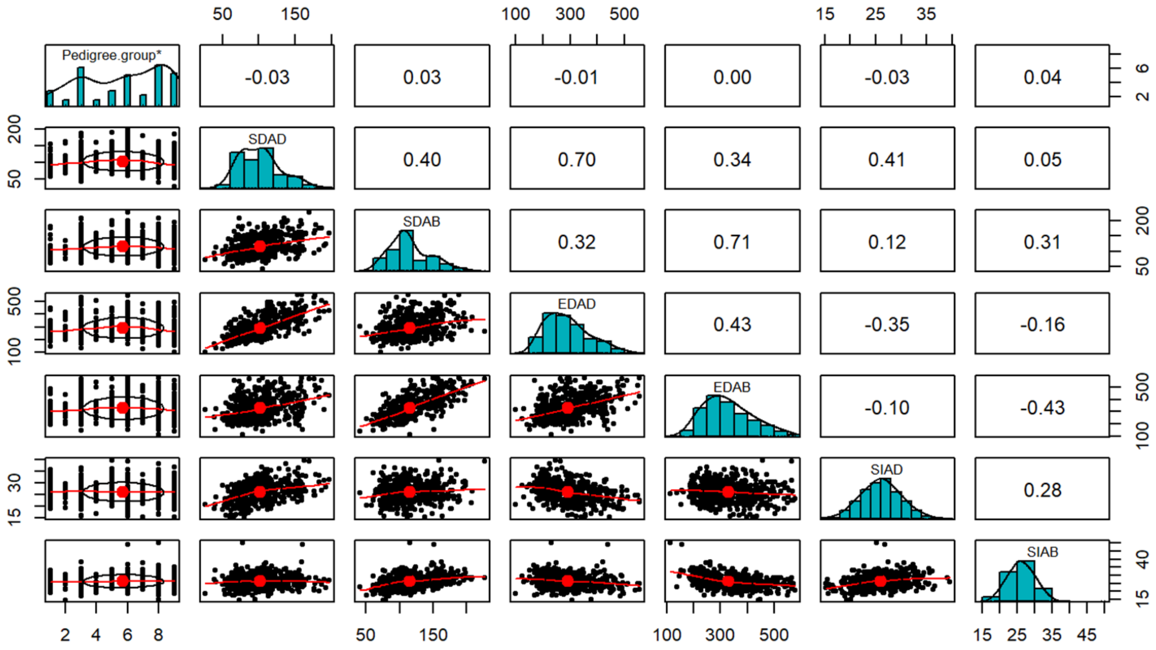

Figure 3. 3 Correlation between anatomical variables adaxial stomatal density (SDAD), abaxial stomatal density (SDAB), adaxial epidermal cell density (EDAD), abaxial epidermal cell density (EDAB), adaxial stomatal index (SIAD), and abaxial stomatal index (SIAB) in the canola accessions. [The value inside the box represents Pearson correlation coefficient].

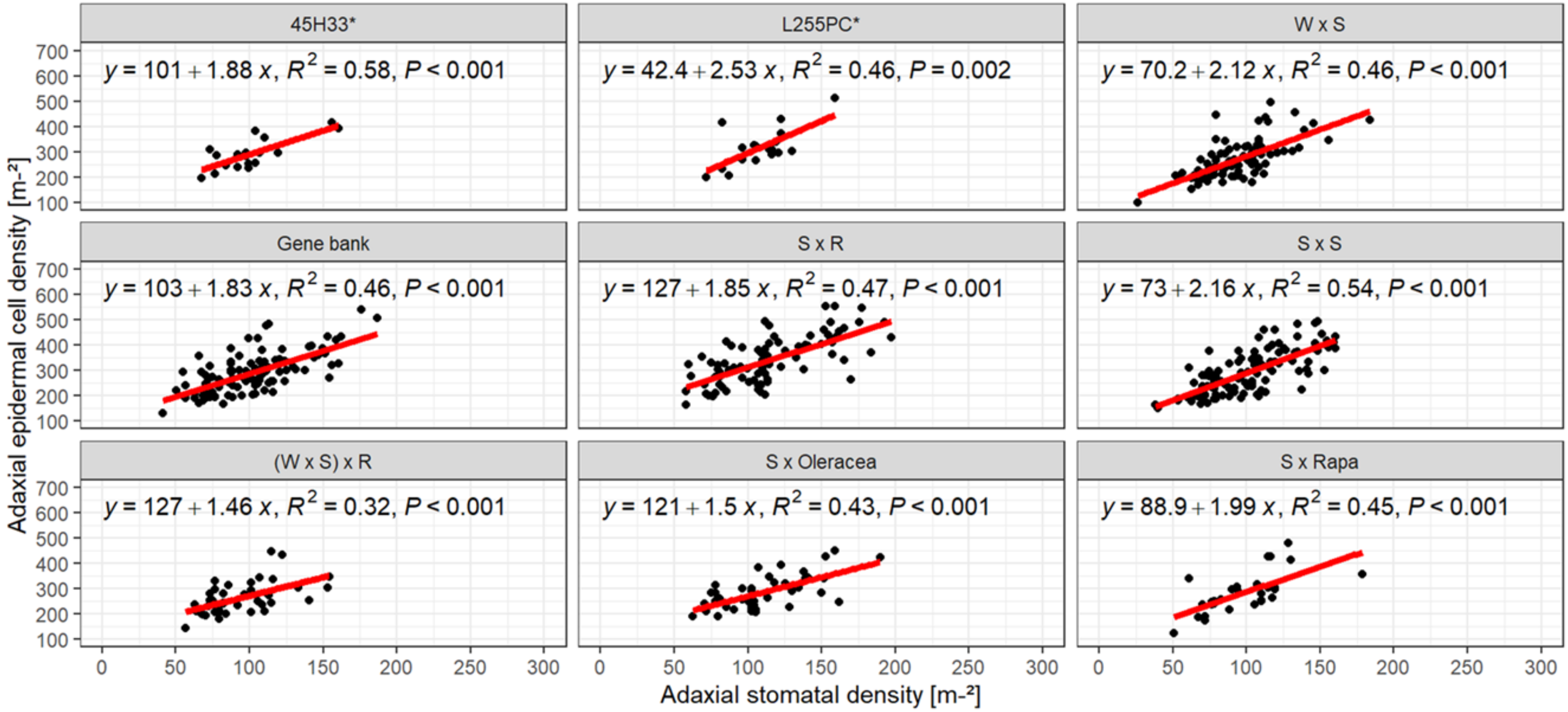

Figure 3. 4 A linear relationship between adaxial epidermal cell density and adaxial stomatal density across different canola pedigree groups. Points represent values and the line represents a fitted linear regression [(winter canola × spring canola) × rutabaga ((W × S) × R), spring canola × *Brassica oleracea* (S × Oleracea), spring canola × rutabaga (S × R), spring canola × *Brassica rapa* (S × Rapa) , spring canola × spring canola (S × S), and winter canola × spring canola (W × S) crosses, as well as accessions collected from The Plant Gene Resources of Canada, Saskatoon (Gene bank)]. * Indicates Checks [L255PC (LibertyLink) & 45H33 (Roundup Ready)].

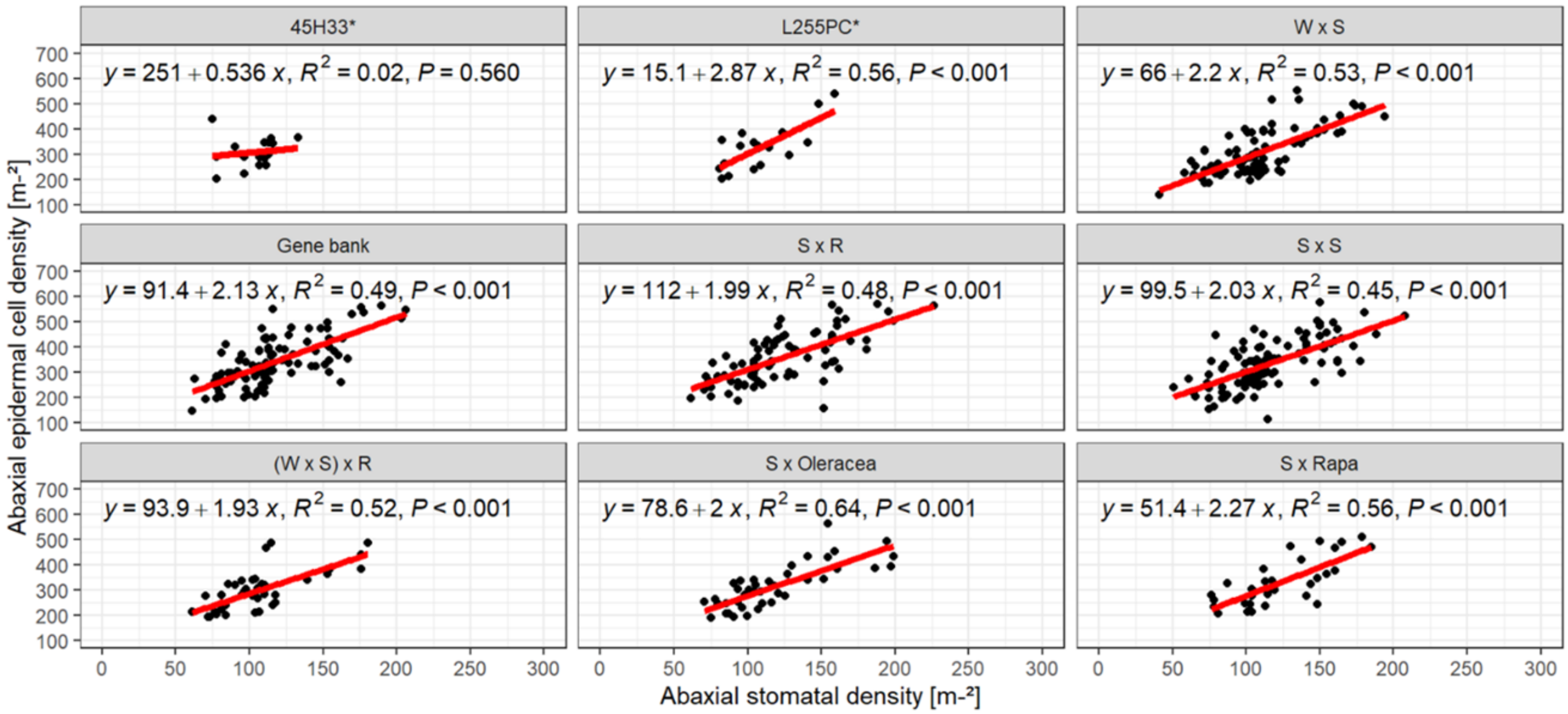

Figure 3. 5 A linear relationship between abaxial epidermal cell density and abaxial stomatal density across different canola pedigree groups. Points represent values and the line represents a fitted linear regression. [(winter canola × spring canola) × rutabaga ((W × S) × R), spring canola × *Brassica oleracea* (S × Oleracea), spring canola × rutabaga (S × R), spring canola × *Brassica rapa* (S × Rapa) , spring canola × spring canola (S × S), and winter canola × spring canola (W × S) crosses, as well as accessions collected from The Plant Gene Resources of Canada, Saskatoon (Gene bank)].* indicates Checks [L255PC (LibertyLink) & 45H33 (Roundup Ready)].

Table 3. 3 Canola accessions that significantly outperformed the checks for adaxial stomatal density (SDAD), abaxial stomatal density (SDAB), adaxial epidermal cell density (EDAD), abaxial epidermal cell density (EDAB), and adaxial stomatal index (SIAD) [* significantly higher than check 1 (45H33); **significantly higher than check 2 (L255PC); no star: significantly higher than both checks. [(winter canola × spring canola) × rutabaga ((W × S) × R), spring canola × *Brassica oleracea* (S × Oleracea), spring canola × rutabaga (S × R), spring canola × *Brassica rapa* (S × Rapa) , spring canola × spring canola (S × S), and winter canola × spring canola (W × S) crosses, as well as accessions collected from The Plant Gene Resources of Canada, Saskatoon (Gene bank)].

Canola pedigree groups	SDAD	SDAB	EDAD	EDAB	SIAD
(W × S) × R	None	None	None	1CA2160.017-A2069**	None
Gene bank	None	Westar**	None	Westar**	Golden (non-CQ)
S × Oleracea	5CA1678.401-A2090, 5CA1679.821-A2099	5CA1358.1469-A2099**, 5CA1679.795-A2099, 5CA1679.821-A2099, 5CA1679.825-A2090	None	5CA1358.1446-A2099	5CA1678.396-A2099
S × R	1IA2519.005-A2000, 1CA2336.142-A2050, 1CA2337.090-A2050, 1CA2354.144-A2070	1CA2348.038-A1000, 1CA2383.038-A2070, 1CA2336.122-A2070, 1IA2519.005-A2000, 1CA2354.144-A2070, 1CA2334.257-A2070, 1CA2342.072-A2070, 1CA2354.063-A2070, 1CA2337.090-A2050	1CA2354.063-A2070, 1IA2519.005-A2000, 1CA2334.273-A2070	1CA2342.074-A2070**, 1CA2348.038-A1000**, 1CA2383.038-A2070**, 1CA2354.144-A2070,1IA 2519.005-A2000, 1CA2342.072-A2070, 1CA2336.120-A2070	1CA2337.090-A2050
S × S	A04-74NA**	A03-21367NI**, Alto, Q2	02-24557-4*	A04-73NA, Alto	A04-75NA*, Conquest, A01-104NA
W × S	None	1RA1638.102, 1CA1609.060-A2069	A07-35NI*	1RA1638.102, 1CA1609.060-A2069	None

3.4 Discussion

The examination of SDAD, SDAB, EDAD, and EDAB unveiled intriguing patterns across different canola pedigree groups. Notably, variation between the pedigree groups existed for these traits; among these, the S × R pedigree group exhibited the highest stomatal and epidermal cell densities suggesting the potential genetic control of these traits. Stomata regulate gas exchange, including the uptake of carbon dioxide for photosynthesis and the release of oxygen and water vapor. Higher stomatal density can enhance the plant's ability to exchange gases with the atmosphere, potentially leading to increased photosynthetic rates, which means increased source strength and improved water use efficiency. These findings align with the previous reports highlighting the genetic regulation of stomatal development and density in plants (Hepworth et al., 2018). Epidermal cells play essential roles in protecting the plant from environmental stresses, regulating water loss through transpiration, and serving as a barrier against pathogens and pests. Higher epidermal cell density can enhance leaf defense mechanisms (Karabourniotis et al., 2020). Trichome density, while not present in all canola accessions, demonstrated notable variations in canola pedigree groups. Higher proportion of accessions belonging to the (W × S) × R and S × Oleracea pedigree groups had trichomes. Higher trichome density in canola plants has multiple physiological benefits, such as reduced water loss, enhanced pest resistance, better temperature regulation and UV protection. Gruber et al. (2006) also reported several benefits of trichomes in canola, and this include enhanced resistance against pests (flea beetles, and aphids). Thus, the canola accessions carrying trichomes identified from this thesis research can be used in breeding to develop insect pest resistant canola cultivars. Correlation and regression analyses elucidated the relationships between anatomical variables. Strong positive correlations between adaxial and abaxial stomatal densities and epidermal cell densities were observed, indicating coordinated development of these structures. This corroborates the notion that stomatal density is intricately linked with epidermal cell development, influencing gas exchange and transpiration dynamics

(Kusumi et al., 2012). Plant might regulate the number of stomata in relation to the epidermal cell density to optimize important physiological processes such as gas exchange, transpiration, and water-use efficiency. The positive correlation suggests a coordinated regulation between these two features, indicating that canola accessions might be fine-tuning their structure to maintain an optimal balance between gas exchange and structural support. Emmeans comparisons highlighted several canola accessions that outperformed both checks for these anatomical variables (Table 3.3). This indicates the potential for selecting canola accessions carrying these favorable anatomical traits for use in breeding programs. Canola accessions outperforming the checks for SDAD and SDAB along with increased EDAD and EDAB have enhanced leaf structure and perhaps superior physiological processes such as photosynthesis, gas exchange, stomatal conductance resulting in better resource partitioning from source to sink organs (e.g. yields).

Overall, this study provides valuable insights into the morphological diversity of canola leaves in different canola pedigree groups. High stomatal density, such as that observed in the accessions Wester (SDAB, EDAB) from the Gene Bank pedigree group, and 5CA1679.821-A2099 (SDAD, SDAB) from S × Oleracea pedigree group, has the potential to facilitate greater gas exchange, enhancing photosynthetic efficiency and water use efficiency. Similarly, accessions like 1IA2519.005-A2000 (SDAD, SDAB, EDAD, EDAB), 1CA2337. 090.A2050 (SDAD, SDAB, SIAD), and 1CA2354.144-A2070 (SDAD, SDAB, EDAB) from S × R pedigree group exhibit high adaxial and abaxial stomatal and epidermal cell densities, potentially supporting robust photosynthetic performance and improved adaptability to varying environmental conditions. Canola accessions such as Alto (SDAB, EDAB) from S × S pedigree group, and 1RA1638.102 (SDAB, EDAB) and 1CA1609.060-A2069 (SDAB, EDAB) from W × S pedigree group, with high stomatal and epidermal cell densities on both adaxial and abaxial surfaces, potentially demonstrate enhanced physiological traits that contribute to superior growth and yield. These anatomical features play crucial roles in regulating water loss, protecting against herbivory, and optimizing light capture, ultimately enhancing the overall physiological efficiency and resilience of canola plants. Future research could delve deeper into the relationship of stomatal and trichome development with important physiological processes (photosynthesis, transpiration, stomatal conductance) to elucidate their roles in canola physiology and stress resilience.

4.0 General discussion, conclusions, and future prospects

Canola is the third largest oil-producing crop in the world, following soybean and palm oil (USDA, 2022). It is highly valued for its oil content, which is low in saturated fat and high in omega-3 fatty acids, making it a popular choice for both culinary and industrial applications (Canola council of Canada, 2023). A substantial increase in the acreage of canola in the Canadian prairies is a critical topic, as Canada is the world's largest producer of canola. To achieve the desired production levels, focusing on the development of higher-yielding cultivars and implementing enhanced agronomic methodologies is essential (Kirkegaard et al., 2021). Advanced breeding techniques, such as genomic selection and gene editing, hold promise for developing cultivars with improved yield potential, disease resistance, and environmental adaptability (Ton et al., 2020). Additionally, optimizing crop management practices, including precision agriculture, soil health management, and integrated pest management, can further boost productivity. The introduction of herbicide-tolerant and disease-resistant canola cultivars has already revolutionized the industry, leading to significant yield increases and reduced production costs. However, as climate change poses new challenges, such as shifting growing seasons and increased pest pressures, continuous innovation in both breeding and agronomy is necessary (Haile et al., 2014). Furthermore, sustainable practices that reduce environmental impact while maintaining high yields will be key to the long-term viability of canola farming in Canada and beyond (Rampel et al., 2014). Previous improvements in canola cultivars and management practices have contributed significantly to global production, but the potential for further progress through conventional approaches may be reaching its limits, necessitating the adoption of cutting-edge technologies and practices. Alternative strategies for long-term yield enhancement are imperative (Wang et al., 2020) and exploring source-sink relationship and resource partitioning in canola will give the canola industry a new dimension for crop improvement. The anatomical traits of canola leaves in the array of canola pedigree groups assessed will give better insights to breeders who have to develop new approaches to combat abiotic and biotic stresses in a changing climate.

This study highlights the significance of plant morphological and physiological optimization in canola breeding programs, emphasizing the importance of traits related to resource (biomass) allocation and environmental adaptation. In this study, canola accessions carrying superior resource partitioning and source-sink mechanism traits were identified selected 168 canola accessions assessed in this thesis research. They include A01-104NA, 1CA2383.033-A2070, A03-21659NI, 1IA1082.123-A2090, 1RA1488.034, Crusher, Reston, Turret, 1IA1078.145-A2090, IRA1638.084, IRA1638.085, IRA1082.103-A2069, ICA1609.060-A2069, IRA1003.304, Wester, 5CA1679.821-A2099, 1IA2519.005-A2000, 1CA2337.090.A2050, 1CA2354.144-A2070, Alto, 1RA1638.102. Yield was the primary variable, which was a measurement of sink organ performance used to assess the final performance of canola accessions at different sites. Accessions from CDC-north, which had better weather conditions, performed better than the other four site-years indicating the high dependence of yield on the weather conditions. It was also confirmed by other research that yield is highly dependent on the growing season's weather conditions (Sjulgård et al., 2023). The superior performance of canola accessions at CDC-north underscores the importance of favorable weather in maximizing yield through efficient source-sink relationships and resource partitioning which aligns with the existing research (Smith et. al., 2018). Optimal weather conditions enhance photosynthetic activity, improve carbohydrate and nutrient distribution, and reduce environmental stresses, all of which contribute to higher yields (Papantoniou et al., 2013). This illustrates the critical role of environmental factors in agricultural productivity and the necessity of developing strategies to manage and mitigate adverse weather conditions to maintain high crop yields. Similarly, leaf biomass (source organ) was higher in CDC-North sites across all canola pedigree groups indicating that it was environment dependent. In CDC-North (2023), notable differences in thousand seed weight (TKW) and yield were observed across various canola pedigree groups, reflecting their diverse genetic backgrounds and responses to environmental factors. Canola accessions such as 1IA1078.145-A2090, 1IA1082.123-A2090, and 1RA1003.304 consistently outperformed both checks for seed yield and TKW at CDC-North (2023), indicative of enhanced seed development and kernel size .The superior performance in TKW and yield underscores the efficient utilization of assimilates and resources by these canola accessions under favorable environmental conditions, contributing to improved crop productivity. This highlights the importance of genetic diversity and adaptive traits in canola breeding programs aimed at enhancing yield potential and resilience across varying agro-climatic regions.

This study also provides a detailed leaf morphological characterization of different canola accessions, highlighting significant variations in leaf architecture with respect to the presence or absence of trichomes, stomata and epidermal cell densities, which can influence overall plant performance and adaptability. Trichomes, important in plant defence mechanism was present in most canola accessions indicating a strong defense system against the harsh environment and pest management (Alahakoon et al., 2016). It was an important finding since trichome rich canola cultivars are becoming popular in North America for extra benefits like protection against flea beetles (Gavloski, 2017; Soroka et al., 2007). The positive correlation between stomatal density and epidermal cell density from this study provide insights into plant adaptation strategies and help researchers develop crops with improved water use efficiency and stress tolerance which agree with the existing research (Verma et al., 2020). There are canola accessions with significantly high biomass, yield, and a high density of trichomes and stomata. This study found that certain canola accessions exhibited superior source-sink relationships alongside advantageous leaf morphological traits. These superior canola accession include, 1CA1609.060-A2069 with significantly higher thousand seed weight, stomatal density and epidermal cell density; and

1CA2354.144-A2070 with significantly higher yield, stomatal density, epidermal cell density. The presence of such canola accessions implies that canola breeding programs can focus on these traits to develop more resilient and productive new canola cultivars. These canola accessions can ensures efficient photosynthesis, effective defense mechanisms, and better adaptation to environmental stresses.

The following conclusions were drawn from this research study:

- Optimum weather conditions promote higher biomass production of canola accessions.
- The functioning of both source and sink organs is strongly influenced by prevailing weather conditions, underscoring the critical role of environmental factors in regulating plant productivity and resource allocation.
- Despite belonging to the same pedigree groups, canola accessions did not perform consistently, highlighting variability in their response to environmental conditions, genetic factors, and management practices.
- More resources were partitioned towards the sink organs of canola accessions 1CA2383.03-A2070 (S × R), A03-21659NI (S × S), 1IA1082.123-A2090 (W × S), 1RA1488.034 (W × S) with higher yield than checks.
- Trichomes are prevalent among most of the canola accessions including the checks.
- A direct correlation exists between the density of stomata and epidermal cells, suggesting a coordinated development of leaf anatomy for structural support.
- Canola accessions derived from different pedigrees varied in epidermal cell density, and stomatal density supporting the second hypothesis of this research.

Superior canola accessions identified in this research can be used in the Canola Breeding Program, University of Alberta to breed for increased yields, biotic and abiotic stresses in collaborations with seed companies. This collaboration will facilitate the integration of newfound knowledge, thereby contributing to the ongoing evolution and improvement of the canola yields to reach the 52 bu/acre target set by the canola council of Canada.

Some future research areas to further utilize the superior canola accessions identified and knowledge gained from this thesis research include:

- Germplasm expansion: Superior canola accessions (with more resource partitioning abilities) from this research can be a base for collecting and characterizing diverse canola germplasm that can result in newer trait and a broad gene pool can be created.
- Genome editing: New canola accessions can be developed by removing undesired traits using clustered regularly interspaced short palindromic repeats (CRISPR) tool from selected material in this study.
- Genetic analysis and mapping to pinpoint the specific genes responsible for desirable traits in the identified canola accessions with high yields and higher biomass allocation.
- Development of molecular markers linked to these specific genes will allow for efficient selection during breeding.
- Enhancing physiological processes: Study of the stomatal density can be additionally correlated with stomatal conductance, water use efficiency, photosynthesis to select the accessions with better performance.
- Plant-pathogen interactions: Extensive research can be done to investigate how trichomes influence plant defense against pathogens.

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Appendices

Supplementary Table 2. 1 List of 168 *B. napus* accessions and the two checks 45H33 and L255PC used in the field experiment.

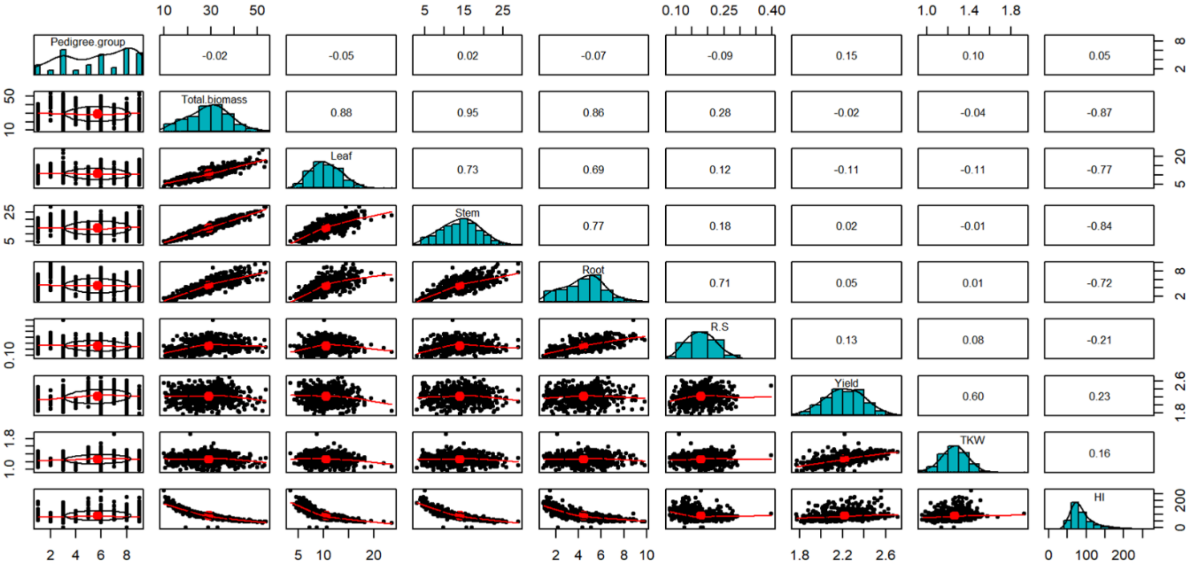
Accession number	Pedigree group*	Old registration number	New registration number
1	(W × S) × R	1CA2639.008-A1230	1CA2639.008-A2040
2	(W × S) × R	1CA2416.043-A1260	1CA2416.043-A2070
3	(W × S) × R	1RA1954.217-A1257	1RA1954.217-A2068
4	(W × S) × R	1CA2165.067-A1259	1CA2165.067-A2069
5	(W × S) × R	1RA2054.102-A1208	1RA2054.102-A2008
6	(W × S) × R	1CA2591.004-A1230	1CA2591.004-A2040
7	(W × S) × R	1CA2582.010-A1230	1CA2582.010-A2040
8	(W × S) × R	1RA1751.287-A1277	1RA1751.287-A2088
9	(W × S) × R	1CA2165.063-A1259	1CA2165.063-A2069
10	(W × S) × R	1CA1846.011-A1208	1CA1846.011-A2009
11	(W × S) × R	1CA2160.017-A1259	1CA2160.017-A2069
12	(W × S) × R	1CA2598.005-A1230	1CA2598.005-A2040
13	(W × S) × R	1CA2631.006-A1230	1CA2631.006-A2040
14	(W × S) × R	1RA1751.288-A1277	1RA1751.288-A2088
15	Gene bank	Regent	Regent
16	Gene bank	Nugget	Nugget
17	Gene bank	Golden (non-CQ)	Golden (non-CQ)
18	Gene bank	Pivot	Pivot
19	Gene bank	OAC Summit	OAC Summit
20	Gene bank	Tower	Tower
21	Gene bank	OAC Springfield	OAC Springfield
22	Gene bank	Apollo (LL)	Apollo (LL)
23	Gene bank	Stallion (TR tolerant)	Stallion (TR tolerant)
24	Gene bank	AC Tristar	AC Tristar
25	Gene bank	AC Elect	AC Elect
26	Gene bank	Midas	Midas
27	Gene bank	Topas	Topas
28	Gene bank	Option 501	Option 501
29	Gene bank	Prominent	Prominent
30	Gene bank	Oro	Oro
31	Gene bank	Next 500	Next 500
32	Gene bank	OAC Triumph	OAC Triumph
33	Gene bank	Agassiz	Agassiz
34	Gene bank	Zephyr	Zephyr
35	Gene bank	Reston	Reston
36	Gene bank	Profit	Profit
37	Gene bank	Turret	Turret
38	Gene bank	AC Excel	AC Excel
39	Gene bank	Argentine	Argentine
40	Gene bank	Next 700	Next 700
41	Gene bank	Westar	Westar
42	Gene bank	Hylite 201 (Apetalous)	Hylite 201 (Apetalous)
43	Gene bank	Vanguard	Vanguard
44	Gene bank	Crusher	Crusher

45	Gene bank	Tanka	Tanka
46	Gene bank	Target	Target
47	Gene bank	Senator	Senator
48	Gene bank	Tribute	Tribute
49	Gene bank	OAC Triton	OAC Triton
50	S × Oleracea	5CA1678.393-A1298	5CA1678.393-A2099
51	S × Oleracea	5CA1300.480-A1298	5CA1300.480-A2099
52	S × Oleracea	5CA1677.475-A1298	5CA1677.475-A2099
53	S × Oleracea	5CA1358.1469-A1298	5CA1358.1469-A2099
54	S × Oleracea	1RA1743.028	1RA1743.028
55	S × Oleracea	5CA1679.825-A1290	5CA1679.825-A2090
56	S × Oleracea	5CA1677.483-A1298	5CA1677.483-A2099
57	S × Oleracea	5CA1679.795-A1298	5CA1679.795-A2099
58	S × Oleracea	5CA1678.401-A1290	5CA1678.401-A2090
59	S × Oleracea	5CA1679.801-A1298	5CA1679.801-A2099
60	S × Oleracea	5CA1358.1446-A1298	5CA1358.1446-A2099
61	S × Oleracea	5CA1679.821-A1298	5CA1679.821-A2099
62	S × Oleracea	5CA1358.1463-A1298	5CA1358.1463-A2099
63	S × Oleracea	5CA1678.396-A1298	5CA1678.396-A2099
64	S × R	1CA2337.048-A1260	1CA2337.048-A2070
65	S × R	1CA2383.038-A1260	1CA2383.038-A2070
66	S × R	1CA2354.097-A1260	1CA2354.097-A2070
67	S × R	1CA2336.122-A1260	1CA2336.122-A2070
68	S × R	1CA2506.040-A1230	1CA2506.40-A2040
69	S × R	1IA2522.011-A1200	1IA2522.011-A2000
70	S × R	1CA2335.105-A1240	1CA2335.105-A2050
71	S × R	1CA2342.091-A1240	1CA2342.091-A2050
72	S × R	1IA2517.006-A1200	1IA2517.006-A2000
73	S × R	1CA2342.074-A1260	1CA2342.074-A2070
74	S × R	1CA2337.046-A1260	1CA2337.046-A2070
75	S × R	1CA2342.072-A1260	1CA2342.072-A2070
76	S × R	1CA2343.079-A1240	1CA2343.079-A2050
77	S × R	1CA2336.120-A1260	1CA2336.120-A2070
78	S × R	1CA2335.080-A1260	1CA2335.080-A2070
79	S × R	1CA2348.038-A1208	1CA2348.038-A1000
80	S × R	1CA2343.052-A1260	1CA2343.052-A2070
81	S × R	1CA2354.063-A1260	1CA2354.063-A2070
82	S × R	1CA2335.069-A1260	1CA2335.069-A2070
83	S × R	1IA2519.005-A1200	1IA2519.005-A2000
84	S × R	1CA2383.033-A1260	1CA2383.033-A2070
85	S × R	1CA2355.092-A1260	1CA2355.092-A2070
86	S × R	1CA2334.273-A1260	1CA2334.273-A2070
87	S × R	1CA2335.095-A1260	1CA2335.095-A2070
88	S × R	1CA2334.257-A1260	1CA2334.257-A2070
89	S × R	1CA2354.144-A1260	1CA2354.144-A2070
90	S × R	1CA2337.090-A1240	1CA2337.090-A2050
91	S × R	1CA2336.142-A1240	1CA2336.142-A2050
92	S × Rapa	5CA1627.1537-A1280	5CA1627.1537-A2090
93	S × Rapa	5CA1627.1563-A1280	5CA1627.1563-A2090
94	S × Rapa	5CA1625.272-A1260	5CA1625.272-A2070

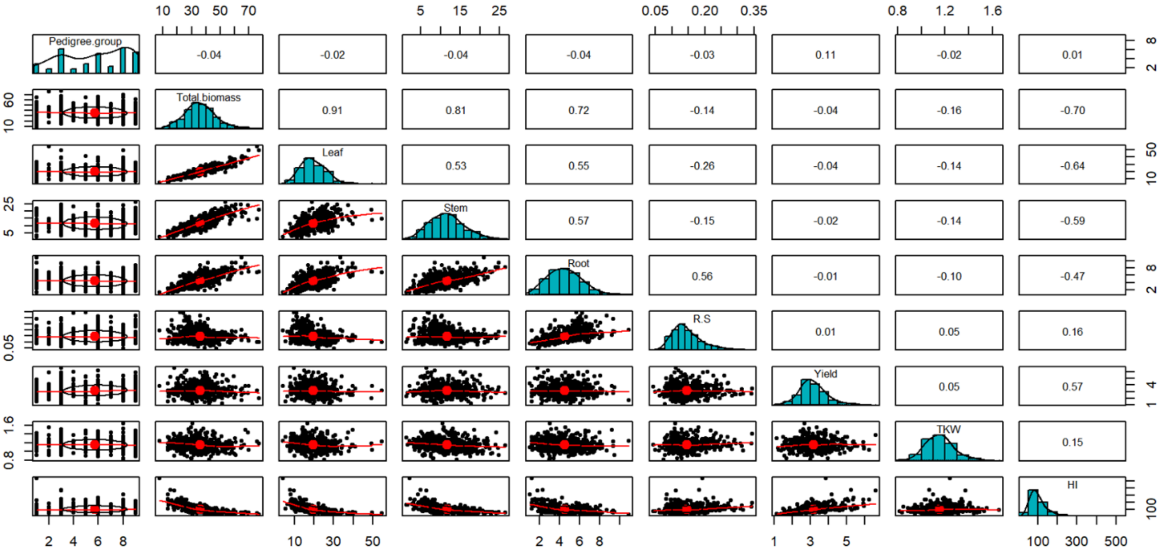
95	S × Rapa	5CA1627.1573-A1280	5CA1627.1573-A2090
96	S × Rapa	5CA1627.1704-A1280	5CA1627.1704-A2090
97	S × Rapa	5CA1627.1529-A1280	5CA1627.1529-A2090
98	S × Rapa	5CA1627.1533-A1280	5CA1627.1533-A2090
99	S × Rapa	5CA1627.1548-A1280	5CA1627.1548-A2090
100	S × Rapa	5CA1627.1576-A1280	5CA1627.1576-A2090
101	S × Rapa	5CA1627.1549-A1280	5CA1627.1549-A2090
102	S × S	Peace	Peace
103	S × S	Kelsey	Kelsey
104	S × S	A03-21449NI PM1+2	A03-21449NI
105	S × S	Cougar	Cougar
106	S × S	A06-20NA	A06-20NA
107	S × S	Hi-Q	Hi-Q
108	S × S	A04-72NA (A02-22536)	A04-72NA
109	S × S	UA BountyGold (A05-17NI)	UA BountyGold
110	S × S	A03-22808NA	A03-22808NA
111	S × S	02-24557-4	02-24557-4
112	S × S	Altex-1	Altex-1
113	S × S	Roper	Roper
114	S × S	A06-19NA	A06-19NA
115	S × S	A05-22NA (A03-22564NA)	A05-22NA
116	S × S	SILEX (A91-15026)	SILEX
117	S × S	A07-28NA	A07-28NA
118	S × S	A04-74NA (A02-22561)	A04-74NA
119	S × S	A03-22762NA	A03-22762NA
120	S × S	A04-73NA (A02-22606 NA)	A04-73NA
121	S × S	A03-22620NA	A03-22620NA
122	S × S	A03-22307NA	A03-22307NA
123	S × S	A05-6NI	A05-6NI
124	S × S	Conquest	Conquest
125	S × S	A03-21367NI PM1+2	A03-21367NI
126	S × S	Quantun	Quantun
127	S × S	Alto	Alto
128	S × S	A07-26NR	A07-26NR
129	S × S	A05-4NI (A03-21821NI PM1+2)	A05-4NI
130	S × S	Q2	Q2
131	S × S	Andor	Andor
132	S × S	A03-22758NA	A03-22758NA
133	S × S	A05-10NI (A03-21292NI PM1+2)	A05-10NI
134	S × S	A01-104NA	A01-104NA
135	S × S	1CA0110.004-A2006	1CA0110.004
136	S × S	A04-75NA (A02-22707)	A04-75NA
137	S × S	A03-22805NA	A03-22805NA
138	S × S	A03-21659NI PM1+2	A03-21659NI
139	W × S	1IA1082.121-A1280	1IA1082.121-A2090
140	W × S	1RA1003.304	1RA1003.304
141	W × S	1RA1638.084	1RA1638.084
142	W × S	1RA1584.140	1RA1584.140
143	W × S	1CA1609.060-A1259	1CA1609.060-A2069
144	W × S	1RA1498.016	1RA1498.016

145	W × S	UA AlfaGold (A07-29NI)	UA AlfaGold
146	W × S	1IA0944.317-A4090	1IA0944.317-A2099
147	W × S	1RA1484.038	1RA1484.038
148	W × S	1IA1082.118-A1280	1IA1082.118-A2090
149	W × S	1CA1609.061-A1259	1CA1609.061-A2069
150	W × S	1RA1498.014	1RA1498.014
151	W × S	1CA1616.198-A1259	1CA1616.198-A2069
152	W × S	1IA1078.145-A1280	1IA1078.145-A2090
153	W × S	1CA1618.134-A1259	1CA1618.134-A2069
154	W × S	1IA1082.123-A1280	1IA1082.123-A2090
155	W × S	1IA1078.128-A1270	1IA1078.128-A2080
156	W × S	1RA1638.100	1RA1638.100
157	W × S	1IA1082.103-A1270	1IA1082.103-A2080
158	W × S	1IA1078.137-A1270	1IA1078.137-A2080
159	W × S	1RA1638.102	1RA1638.102
160	W × S	1CA1616.210-A1259	1CA1616.210-A2069
161	W × S	1RA1638.085	1RA1638.085
162	W × S	1IA0944.312-A1297	1IA0944.312-A4090
163	W × S	1IA1078.155-A1280	1IA1078.155-A2090
164	W × S	A07-35NI	A07-35NI
165	W × ×S	1RA1143.141	1RA1143.141
166	W × S	1RA1488.034	1RA1488.034
167	W × S	1CA0591.361-A1299	1CA0591.361
168	W × S	1RA1488.014	1RA1488.014
169	45H33	Check	45H33
170	L255PC	Check	L255PC

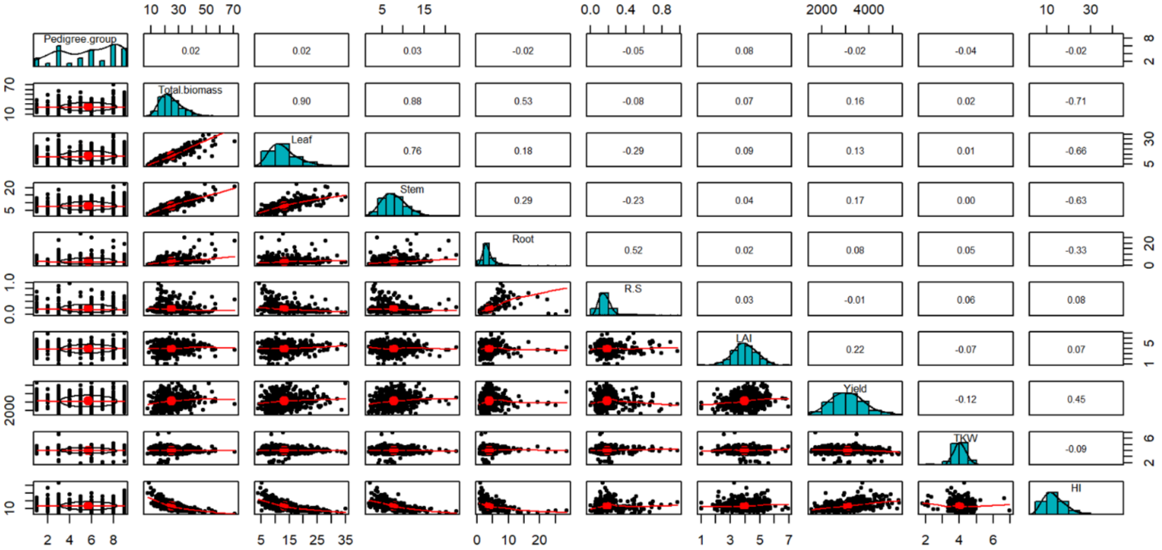
**see pedigree group abbreviation in the plant material section*



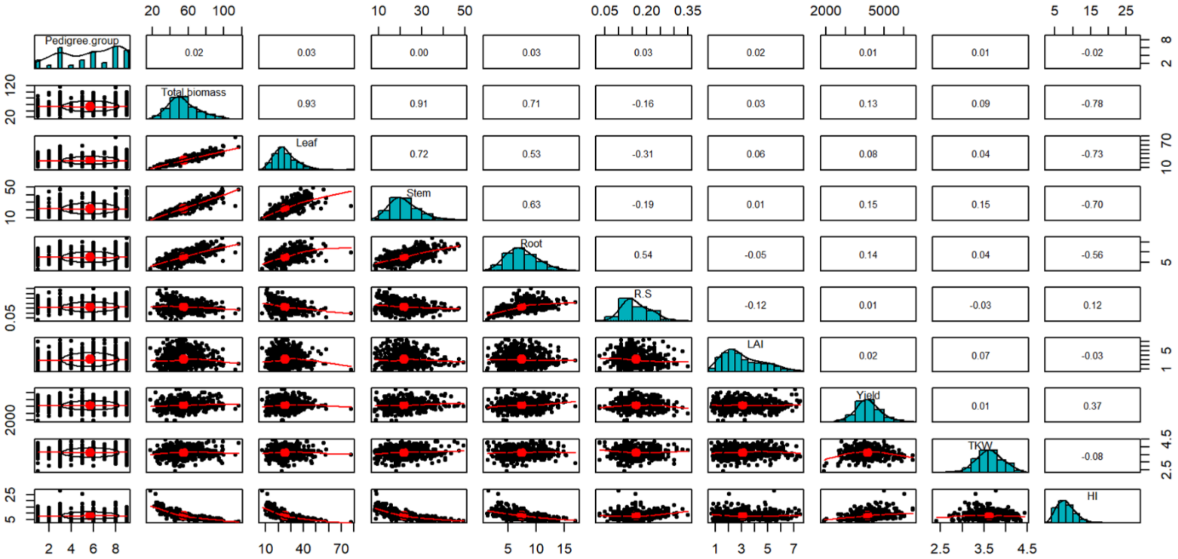
Supplementary Figure 2. 1 Correlation graph of site West-240 (2021) within total biomass, leaf biomass (leaf), stem biomass (stem), root biomass (root), root shoot ratio (R/S), yield, thousand seed weight (TKW), harvest index (HI) in the canola accessions. The value inside the box represents the Pearson correlation coefficient.



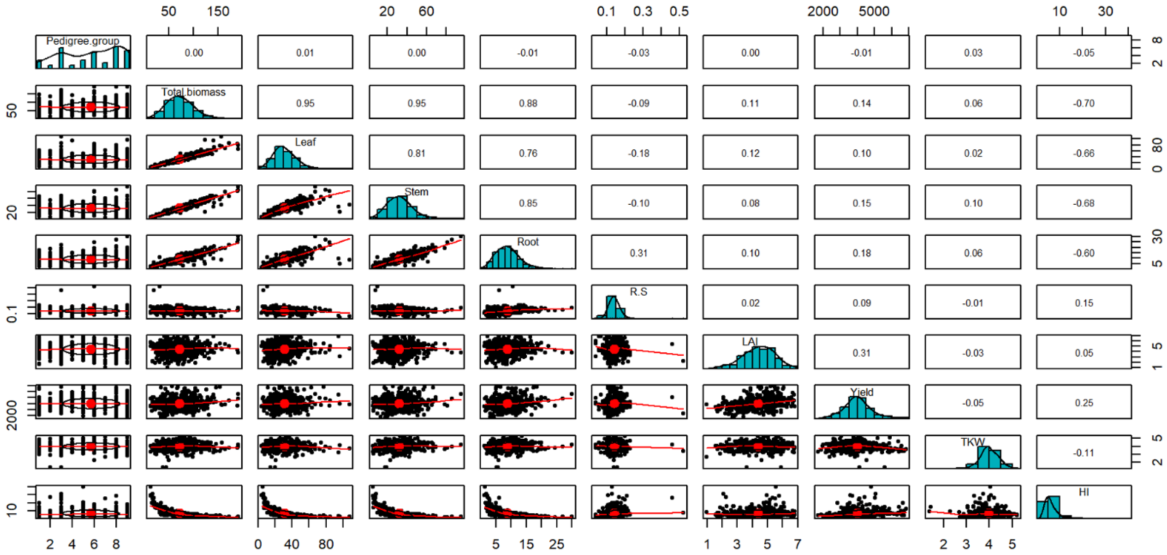
Supplementary Figure 2. 2 Correlation graph of site St. Albert (2022) within total biomass, leaf biomass (leaf), stem biomass (stem), root biomass (root), root shoot ratio (R/S), yield, thousand seed weight (TKW), harvest index (HI) in the canola accessions. The value inside the box represents the Pearson correlation coefficient.



Supplementary Figure 2. 3 Correlation graph of site West-240 (2023) within total biomass, leaf biomass (leaf), stem biomass (stem), root biomass (root), root shoot ratio (R/S), leaf area index (LAI), yield, thousand seed weight (TKW), harvest index (HI) in the canola accessions. The value inside the box represents the Pearson correlation coefficient.



Supplementary Figure 2. 4 Correlation graph of site St. Albert (2023) within total biomass, leaf biomass (leaf), stem biomass (stem), root biomass (root), root shoot ratio (R/S), leaf area index (LAI), yield, thousand seed weight (TKW), harvest index (HI) in the canola accessions. The value inside the box represents the Pearson correlation coefficient.



Supplementary Figure 2. 5 Correlation graph of site CDC-N (2023) within total biomass, leaf biomass (leaf), stem biomass (stem), root biomass (root), root shoot ratio (R/S), leaf area index (LAI), yield, thousand seed weight (TKW), harvest index (HI) in the canola accessions. The value inside the box represents the Pearson correlation coefficient.