# GENOME-WIDE ASSOCIATION STUDY AND GENOMIC PREDICTION FOR POD SHATTER RESISTANCE IN *BRASSICA NAPUS* L.

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**Title**

Genome-Wide Association Study and Genomic Prediction for Pod Shatter Resistance in *Brassica napus* L.

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

**MASTER OF SCIENCE**

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## ABSTRACT

The release of seeds from a mature dry pod is known as shattering. Pod shattering results in significant yield losses in rapeseed/canola. An experiment was conducted in five environments in 2020 and 2021 under the field and the greenhouse conditions using 150 spring-type *Brassica napus* genotypes. Genome-wide association study (GWAS) identified 21 significant SNPs for pod shattering using eight different GWAS models. The phenotypic variation explained by these SNPs ranged from 3.8 -25.4%. Three potential candidate genes, *IND*, *AGL65,* and *MAN7*, were identified for the pod shattering. Genomic prediction was done using 14 genomic selection models. The prediction ability for pod shatter resistance ranged from 0.18 – 0.50. This study suggested that at least one locus on chromosome A09 is associated with pod shattering. The study also suggests that genomic selection has the potential to select pod shatter resistance germplasm at an early stage to use in the breeding program.

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# CHAPTER 1. GENERAL INTRODUCTION

Rapeseed (*Brassica napus* L., genome AACC, 2n = 4x = 38) is an amphidiploid species of Brassicaceae family. It originated in the Mediterranean region through a spontaneous hybridization between turnip rape (*Brassica rapa* L., genome AA, 2n = 2x = 20) and cabbage (*Brassica oleracea* L., genome CC, 2n = 2x = 18) followed by chromosome doubling. This resulted in rapeseed about 10,000 years ago (U, 1935; Raman et al., 2014; Assefa et al., 2018). As an oilseed crop, rapeseed was domesticated about 400–500 years ago (Raman et al., 2014). Rapeseed with natural oil quality, contains high erucic acid, often used for industrial purposes. A rapeseed cultivar with low erucic acid (<2%) and glucosinolate (<30 μmol $g^{-1}$) was identified by Canadian plant breeders at the University of Manitoba (Mag, 1983; Lin et al., 2013). They named it canola which stands for Canada oil or Canada oil with low acid (Assefa et al., 2018).

Rapeseed/canola is an important oilseed crop in the world. It is the world's second-largest edible oilseed crop after soybean (*Glycine max* L.) (Huang et al., 2016). It grows extensively in temperate zones including Northern America, Northern Europe, and Asia (Morgan et al., 2000). In 2019, 3.6 million tons of canola were produced in the United States which is 5.2% of global canola production (USDA-NASS, 2019; FAO, 2020; Secchi et al., 2021). North Dakota harvested 83% of U.S. canola in 2019 (Secchi et al., 2021). While North Dakota is in the lead of canola production, other states such as Minnesota, Oklahoma, Kansas, Texas, Montana, Idaho, Oregon, Washington, and Kentucky also produce rest of the 17% canola in the United States.

Canola has both spring and winter type growth habits. It is a cool-season broadleaf crop. Winter canola planted between August and November. After overwintering over the winter, it resumes growth in spring. Spring canola does not need overwintering which is usually planted after March (Assefa et al., 2018). Most of the canola harvested in the U.S. are spring canola

(Secchi et al., 2021), while winter canola is more widely grown in Europe than spring canola (Steponavičius et al., 2019). Winter canola can produce 20-30% more yield than spring canola, but the survival of winter canola is low and inconstant in the northern United States which limits the yearly production of winter canola in the U.S. (Chao et al., 2021).

Pod shattering, in general, refers to the release of the seeds from a dry fruit upon maturity. The process of shattering differs among crops. In crucifers and legumes, it refers to the opening of the pod or the silique. In cereals, it refers to the detachment of the fruit from the pedicel (Ogutcen et al., 2018). Different anatomical structures and mechanisms are involved in the process of shattering. Fruit dehiscence is a natural process of seed dispersal, but in cultivated crops it is one of the major sources of yield loss. For this reason, in crop domestication, the indehiscence of pods or fruits was likely to be one of the first traits strongly selected.

Arabidopsis (*Arabidopsis thaliana* L), a 'model' plant, belongs to the same family as oilseed rape (Brassicaceae) (Liu et al., 2020). Pod development and structure of *B. napus* are similar to *Arabidopsis thaliana*. Two pod valves joined together by a replum with valve margin cells enclose the seeds inside the pod. When the pods become mature, the valve margin cells separate and detach from the replum causing the seeds to release (Raman et al., 2014). Genetic variation for pod shatter resistance has been identified in *Brassica napus*, *Brassica rapa*, *Brassica juncea,* and *Brassica carinata* (Hu et al., 2012; Raman et al., 2014, 2017; Liu et al., 2016, 2020). Pod shattering intensity differs among *Brassicas* crops (Kaur et al., 2020). *B. carinata*, *B. juncea* and *B. rapa* are less prone to pod shattering than *B. napus* (Kaur et al., 2020) while *B. carinata* reported to be more resistant to pod shattering among these four (Zhang et al., 2016; Raman et al., 2017) species.  Differences in the pod length, water content, vascular bundle

size, cellulose and lignification in the pod walls are responsible for the variations of pod shattering intensity among *Brassicas* crops (Kaur et al., 2020).

When the pods become mature, a dehiscence zone (DZ) developes between the two valves and the replum. In the dehiscence zone, highly differentiated cells weaken the strength of the pods and lead to seed dispersal. Dehiscent fruits make harvesting difficult and lead to significant production losses. For commercial seed production in *Brassica* crops, pod shattering is a highly undesirable trait and causes significant yield losses of up to 70% in rapeseed/canola (Raman et al., 2017; Steponavičius et al., 2019). Pod shattering resistance is not correlated with important agronomic traits such as pod density, length and width of the pod, or seed number per pod (Morgan et al., 2000). The major factors affecting pod shattering resistance are genetic characteristics (Kuai et al., 2016). Genotypes that are susceptible to shattering lose more seed due to pod drop and pod shatter at the preharvest stage (Gulden et al., 2017). Identifying candidate gene(s) associated with pod shattering resistance in the rapeseed/canola germplasm will facilitate a cost-effective marker-assisted selection of desirable alleles in breeding programs.

Multiple genes control pod shatter resistance (Liu et al., 2020; Qing et al., 2021). Several QTLs (quantitative trait loci) have also been identified for pod shatter resistance in oilseed rape (Hu et al., 2012; Raman et al., 2014; Liu et al., 2016). Twelve significant QTLs associated with shatter resistance, together account for about 57% of the genotypic variation, were identified on chromosomes A03, A07, A09, C03, C04, C06, and C08 in the doubled haploid (DH) population from BLN2762/Surpass400 and a diverse panel consisting 181 lines of *B. napus*. (Raman et al., 2014). Six significant QTLs were identified for pod shatter resistance on chromosomes A01, A06, A07, A09, C02, and C05 in a diverse panel of 143 *B. napus*, and bi-parental DH and inter-mated ($IF_2$) populations from R1 (pod shatter resistant, as the maternal parent) and R2 (pod

shatter susceptible, as the paternal parent) (Liu et al., 2016). One consistent locus in the vicinity of *AUXIN RESPONSIVE REGULATOR 18* (*ARR18*) and MADS-box gene *SHATTERPROOF* (*BnShp1*), on linkage group A09, control pod shatter resistance in Australian and Chinese germplasm (Raman et al., 2017). In *Arabidopsis thaliana* and other heterologous systems, several regulatory genes which include *SHATTERPROOF1* (*SHP1*); *SHATTERPROOF2* (*SHP2*); *FRUITFULL* (*FUL*); *INDEHISCENT* (*IND*); *ALCALTRAZ* (*ALC*); and *REPLUMLESS* (*RPL*) are known to control pod shattering (Raman et al., 2017). The expression of valve-margin identity is controlled by *REPLUMLESS* (*RPL*) and the *FRUITFULL* (*FUL*) genes (Kaur et al., 2020). *INDEHISCENT* (*IND*); *ALCALTRAZ* (*ALC*) are involved in several hormonal pathways includes auxin, gibberellins, and ABA biosynthesis which are also important to regulate pod shatter resistance (Raman et al., 2017; and Liu et al., 2020). In *B. oleracea*, *B. napus*, and *B. juncea*, *INDEHISCENT* (*IND*), *PG* (polygalacturonase), and *FRUITFULL* (*FUL*) are important to regulate pod shattering resistance (Kaur et al., 2020). The *SHATTERPROOF* paralogs of *Arabidopsis* (*SHP1* and *SHP2*) are residing in the vicinity of the QTL for pod shatter resistance, in Australian and Chinese oilseed rape, on chromosome A9 (*BnSHP1. A9* and *BnSHP2.A9*) (Liu et al., 2020).

## Objectives

The objectives of this study are:

1. To evaluate a wide collection of *Brassica napus* germplasm accessions for pod shattering resistance both in the greenhouse and in the field conditions.
2. To identify genomic regions in *B. napus* associated with pod shatter resistance by genome-wide association study (GWAS).

3. To identify candidate gene(s) associated with pod dehiscence resistance located around major QTL regions of *B. napus*.
4. To evaluate the effectiveness of genomic prediction (GP) for selecting genotypes for pod shatter resistance in *B. napus*.

# CHAPTER 2. LITERATURE REVIEW

## 2.1. *Brassica* genus

The genus *Brassica* belongs to the family *Brassicaceae*. This family comprises many important crop species. Among them, six species of the genus *Brassica* (*Brassica rapa, B. nigra, B. oleracea, B. juncea, B. napus,* and *B. carinata*) are widely used as oilseed, condiments, fodder or vegetable crops throughout the world. *Brassicaceae* family includes 3709 species and 338 genera. Of the 338 genera, 308 have been further allocated to 44 tribes. One of the most important oil crops is *Brassica napus* L. which belongs to the genus *Brassica* (Cheng et al., 2014).

## 2.2. Domestication of *Brassica napus*

One of the most important attainments of the Neolithic Revolution is plant domestication. About 13,000-10,000 years ago, crop domestication started in the Middle East and the Fertile Crescent, and other regions including South Asia, Mesoamerica, Near Oceania (10,000 years ago), and Eastern North America (6000 years ago). Since the beginning of the domestication, more than 2500 species from about 160 plant families have been domesticated (Ogutcen et al., 2018). *Brassica* is related to *Arabidopsis* and diverged from a common ancestor about 20 million years ago. *Brassica* species are among the oldest cultivated plants and have been cultivated for many years. Since no wild species are known, *Brassica napus* may have arisen (within the past 10,000 years) in cultivation (Raymer 2002; Wang et al., 2011).

## 2.3. Rapeseed and canola

The term “Rapeseed” is used for the oilseeds from both *B. napus* and *B. rapa* species. Canola was developed in 1974, using traditional plant breeding techniques, by researchers from Agriculture and Agri-Food Canada and the University of Manitoba. Later, in 1978, the term

canola (Canadian Oil Low Acid) was trademarked by the Western Canadian Oilseed Crushers Association. Canola specifically indicates rapeseed varieties (*B. napus* and *B. rapa)* if the oil produced by them contain less than 2% erucic acid and meal contain less than 30 μm/gm glucosinolates (Canola Council of Canada 1990). Rapeseed is a self-pollinated crop but 12% to 47% outcrossing can occur depending on the environmental variation.

### 2.4. Growth habit of *Brassica napus*

The species *Brassica napus* is an annual or biennial plant. The flowers are bisexual, pale yellow in color, have four petals and four sepals arranged in a cruciform shape. The flowers also have a pistil, two carpels and six stamens. The stamens are tetradynamous, four long and two short, the outer stamens are shorter than the rest of the stamens. Inflorescences are a raceme, and the flowering begins at the base of the inflorescence. The stems are erect, grow up to 1.5 m, are simple to branched and sparsely hairy and the leaves are waxy. The fruits are silique, cylindrical in shape, about 15 or more seeds per silique arranged in a single row (Gulden et al., 2008).

Canola (*B. napus*) has three types of growth habit around the world: winter, spring and semi-winter. Winter canola is mainly grown in Western Europe while semi-winter canola is grown in China. Spring canola is mainly grown in the USA, Canada, Australia, and India. North Dakota grows spring type canola typically planted and harvested in the same growing season (Wrucke et al., 2018).

### 2.5. Evolution of *Brassica napus*

In general, the *Brassica* crop complex consists of six species; *B. nigra* (black mustard), *B. juncea* (Indian mustard), *B. rapa* (three groups, oleiferous, leafy, and turnip/root forming), *B. napus* (oilseed rape, canola, and root forming/rutabaga), *B. oleracea* (cole crops, i.e., leaf, stem, and flower vegetable crops), and *B. carinata* (Ethiopian mustard).

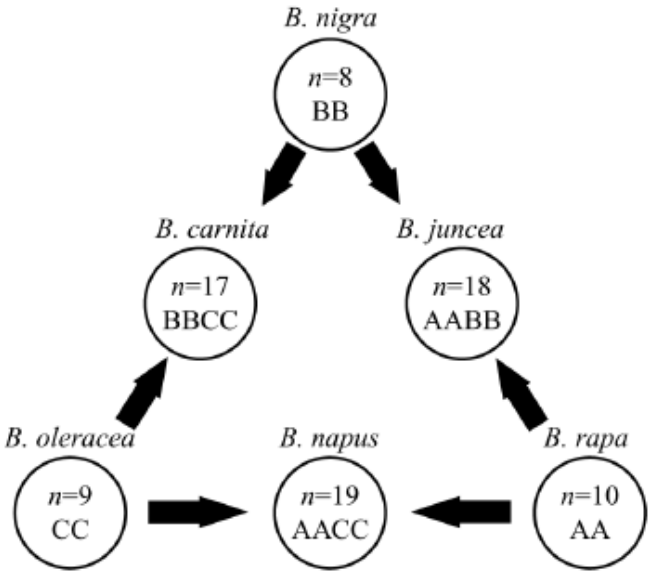


Fig. 2.1. The triangle of U represents the genomic relationship between the six cultivated crop species of *Brassica.*

*Brassica rapa* (n=10), *B. nigra* (n=8), and *B. oleracea* (n=9) are diploid whereas *B. juncea* (n=18), *B. napus* (n=19), *B. carinata* (n=17) are allotetraploids derived from each pair of those three diploid species (U 1935). Crosses between tetraploid and/or diploid plants and microscopic inspection at the synapsis stage of meiosis in these crosses helped to identify the genetic relationships among these six species. A spontaneous hybridization between *B. rapa* (AA, 2n=20) and *B. oleracea* (CC, 2n=18) results in *B. napus* (AACC, 2n= 38). Later this was verified and diagrammatically represented as the triangle of U (Fig. 2.1) by Nagaharu (Raymer 2002).

### 2.6. Pod shattering and the structure associate with shattering

Many plant species dispersed their seed by dehiscing ripped fruit to survive and spread from one place to another. Although this phenomenon is advantageous for some species in nature, dehiscence of siliqua in agriculture results in significant yield loss (Hossain et al 2012). During the domestication process, unlike other crops species, pod shattering has not been

eliminated in cultivated rapeseed. It is an important trait for canola to improve and to get maximum yield.

*B. napus* tends to shed its seed as soon as the pods become fully ripe. Winter *B. napus* cultivars are more resistant to pod shattering than spring cultivars (Haile et al., 2014). Silique shatter can occur both prior to harvest and at harvest. Swathing (or windrowing) and direct harvest (direct combining) are two main methods of harvesting canola. Shattering prior to harvest is due to adverse weather conditions such as strong wind, hail, and frequent changes in temperature and moisture. Shattering at harvest occurs due to the application of external forces such as impact of combine harvesters. Under favorable weather conditions, pre-harvest canola seed loss can reach up to 2.5% which may increase under unfavorable weather conditions. Harvesting beyond the optimal time can cause 20-25% yield losses in canola (Steponavičius et al., 2019).

The detachment of entire organs from a plant is known as abscission while the release of an organ's internal contents is known as dehiscence. With narrow bands of differentiated cells, abscission occurs in the abscission zone and dehiscence occurs in the dehiscence zone (Ogutcen et al., 2018). The fruits of the crucifer family are known as silique. Siliquae are dry dehiscent fruits consisting of two fused carpels that form two locules by a thin and papery white replum. The seeds are enclosed in between the two valves and the valves are attached to the replum forming a suture. The suture is also known as dehiscence zone (DZ) (Fig. 2.2). The siliquae are attached to the raceme by a pedicel at the proximal end from where dehiscence is usually initiated (Hossain et al., 2012).

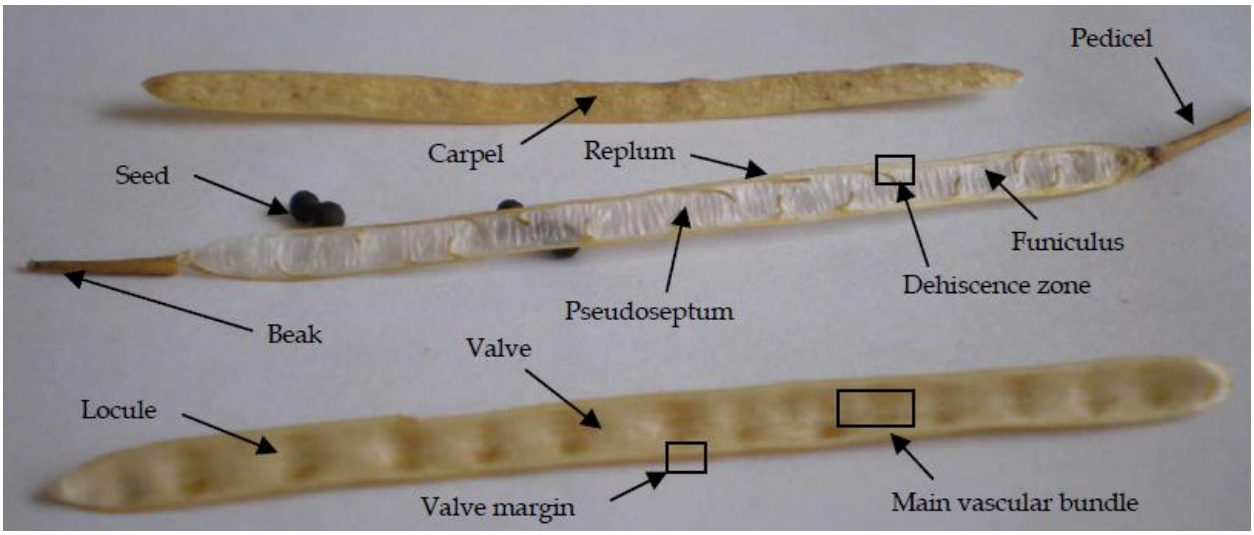


Fig. 2.2. Structural feature of a *Brassica napus* pod (Hossain et al., 2012).

## 2.7. Factors involved in shattering

There are several possible factors (Table 2.1) involved in the siliqua shatter resistance which include morphological, anatomical, physiological, biochemical aspects of siliqua development, and environmental factors.

Table 2.1. Possible factors involved in siliqua shatter

| Factors | Sources | Trait |
|---|---|---|
| Morphological | Siliqua | Siliqua erectness |
| | | Siliqua size, shape and weight |
| | | Density of siliqua |
| | | Pedicel length |
| | Canopy structure | Interaction between plants |
| | Plant | Stem thickness |
| | | Plant height |
| | | Angle of the branches to the main stem |
| | | Number of primary branches |
| Anatomical | Siliqua | Lignification of the suture/dehiscence zone |
| | | Lignification of the siliqua valves |
| | | Size of main vascular bundle |
| | | Size of the dehiscence zone |
| Physiological | Plant | Uniformity of flowering |
| | | Raceme structure |
| Environmental | Abiotic factors | Temperature |
| | | Rain and drought |
| | | Time of sowing |
| | Biotic factors | Pests e.g., siliqua midge, aphids |
| | | Pathogens e.g., Alternaria |

From: (Kadkol et al., 1986; Morgan et al., 1998; Morgan et al., 2003; Summers et al., 2003; Hossain et al., 2012).

### 2.8. Biochemical and molecular basis of shattering

At ripening, highly coordinated and regulated events in growth and differentiation of the dehiscence zone (DZ) and the degradation of the separation layer result in silique dehiscence. Several hormones such as auxin, abscisic acid and ethylene play an important role in the process of silique dehiscence. Cell wall degradation at the DZ, prior to silique desiccation, is triggered by a decrease in auxin and an increase in β-1,4-glucanase (cellulose) activity (Hossain et al., 2012; Ogutcen et al., 2018). Dissolution of middle lamella in the DZ leads to cell separation. When the silique becomes mature, an increased production of ethylene causes hydrolytic enzyme activity at the DZ to increase the enzymatic degradation of cell wall pectins and the middle lamella mediated by cellulose. Cytokinin and gibberellin hormones are also known to be involved in the formation of valve margin but their exact role is yet to be determined (Ogutcen et al., 2018).

### 2.9. Methods for screening pod shatter resistance

Many of the early methods of screening for shatter resistance have been based on visual field observations or manual tests. Due to the difference in maturity, moisture content and environmental conditions, these tests are somewhat subjective and are often not comparable (Hossain et al., 2012). As field evaluation is imprecise, laboratory testing for shattering resistance is needed. Laboratory tests simulate the process of shattering as it occurs under natural conditions (Kadkol 2009). Laboratory evaluation of shattering resistance includes random impact test (RIT), manual bending test, the cantilever test, siliqua twisting, ripping method, pendulum test, and the variable-speed pod splitter (Steponavičius et al., 2019).

Random Impact Test (RIT) (Bruce et al., 2002; Morgan et al., 1998 and 2003; Squires et al., 2003): It measured the breaking response of silique by imitating conditions in the crop canopy caused by agitation during harvest or by poor weather conditions, and estimated the half-life of the sample. This test involves controlled agitation of samples. To achieve constant conditions, the siliques are equilibrated in an atmosphere of constant relative humidity (50%) and temperature (105°C) (Bruce et al., 2002). This method uses a mechanical shaker to shake the pods together with ball bearings in a container. The pods which remain intact are considered resistant to shatter.

Manual bending test: This method evaluates shatter resistance by placing the collected siliqua on a flat surface with angles marked and pedicel held firm. The siliqua is bent anticlockwise. The bending creates stress at which the angle is noted (Hossain et al., 2012).

Cantilever test (Kadkol et al., 1984): This method measures the bending moment and energy required to shatter the siliqua. Here, in a Universal Testing Machine, the siliqua is clamped at the end of the pedicel. To load the siliqua as a cantilever, a steel wedge fixed to the

load cell is used. The applied force is recorded on the chart. The bending moment at the peak of the force displacement graph is defined as shatter resistance.

Siliqua twisting (Tys et al., 2007): This method determines the strength of the dehiscence zone (DZ) by applying twisting force to the siliqua. Using an INSTRON device, torque is applied under a twist of 180° in a holder.

Ripping method (Tan et al., 2007): It measures siliqua dehiscence strength quantitatively at 2.5 cm from pedicel. 6 siliqua per variety kept for 2 weeks with 50% relative humidity and 25°C temperature. At 2.5 cm from the pedicel, a metallic thread laced around the siliqua and laced to the pedicel. Texture analyzer's L-shaped probe lifted the thread, opened the siliqua and recorded opening strength.

Pendulum test (Kadkol et al., 1991; Liu et al., 1994): It measures the energy absorbed by the pendulum during the siliqua shattering process. Here, the siliqua is placed in the machine and the pendulum apparatus strikes the pod with a known force and records the energy absorbed by the siliqua in shattering.

### 2.10. Genes involved in shattering

In *Arabidopsis thaliana*, two MADS-box transcription factors encoding genes *SHATTERPROOF1* (*SHP1*) and *SHATTERPROOF2* (*SHP2*) control pod shattering (Liljegren et al., 2000). Two b-HLH transcription factors, *INDEHISCENT* (*IND*) and *ALCATRAZ* are acting down-stream of and in parallel with *SHP1/2* (Dong and Wang, 2015). During late fruit development, *IND* and *ALC* are specifically expressed in the dehiscence zone (DZ). *IND* acts downstream of *SHP1/2* to control pod shattering (Liljegren et al., 2000, 2004). The *FRUITFULL* (*FUL*) MADS-box gene regulates the valve identity. *FUL* negatively regulates *SHP1/2* expression which determines the expression of *SHP1/2* in the valves (Gu et al., 1998; Ferrándiz

et al., 2000). In addition to *FUL*, *REPLUMLESS* (*RPL*) also restricts the DZ-specific expression of *SHP1/2* and *IND*. *RPL* contributes to the specification of replum identity and encodes a homeodomain transcription factor (Roeder et al., 2003). *NST1* (*NAC SECONDARY WALL THICKENING PROMOTING FACOTR1*) and *SND1* (*SECONDARY WALLASSOCIATED NAC DOMAIN PROTEIN1*, also called *NST3*) are found to be contributed in indehiscent pod (Mitsuda and Ohme-Takagi, 2008; Zhong et al., 2010). *ADPG1* (*ARABIDOPSIS DEHISCENCE ZONE POLYGALACTURONASE1*) and *ADPG2* encode plant specific endo-polygalacturonases (PGs). *ADPG1* and *ADPG2* are expressed in the separation layer of flower organs and fruit DZs and are necessary for silique dehiscence (Ogawa et al., 2009).

In soybean, *Pdh1* (Pod Dehiscence1) gene was identified as one of the candidate genes to be involved in pod shattering (Ralph et al., 2007; Funatsuki et al., 2008). During the lignin deposition, this gene expressed highly in the lignin-rich inner sclerenchyma of pod walls. This activity promotes pod shattering by increasing the torsion of dried pod walls (Funatsuki et al., 2014). NAC (NAM, ATAF1/2 and CUC2) gene *SHATTERING1*-5 (*SHAT1-5*), homologous to *AtNST1/2* which acts as transcriptional activator of secondary cell wall biosynthesis, found to resides in a QTL controlling pod dehiscence in soybean (Dong and Wang, 2015; Dong et al., 2014). In the ventral suture, the excessive lignification of the fiber cap cells (FCCs) is responsible for the indehiscent fruit character in *Glycine max* (Dong et al., 2014).

The loss of fibers in the sutures (“stringless”) is responsible for indehiscent fruit in common bean (*Phaseolus valgaris*) which is controlled by a major QTL, *St* locus. *PvIND1* gene, homologous to *AtIND*, was mapped in a region near the *St* locus. *PvIND1* may not be directly involved in the control of pod dehiscence in common beans which indicate that *PvIND1* may not be the causal gene underlying *St* locus (Gioia et al., 2013; Dong and Wang, 2015).

In pea, a candidate gene was identified on LGIII for pod dehiscence. LGIII is characterized as proline-rich extensin-like protein which is a homolog of peptidoglycan-binding domain protein (PGDB) of *Medicago truncatula* (Tayeh et al., 2015). The *MACE-P015* gene has a peptidoglycan binding function which indicates that this gene might play a critical role in pod dehiscence of pea (Liu et al., 2019).

In rice (*Oryza sativa*), fruit dehiscence is implemented by an abscission layer in between lemma and pedicel. *Shattering4* (*Sh4*), homology to *Myb3*, encodes a transcription factor which involves in the development of a functional abscission layer in the pedicel. A combination of coding and regulatory change of *Sh4* impairs the development of the abscission layer which weakens the shattering phenotype (Li et al., 2006). A major QTL on chromosome 1, underlying gene *qSH1* which is required for formation of the abscission layer in the pedicel, found to control seed shattering in rice (Konishi et al., 2006). *qSH1* encodes a BEL1-type homeobox transcription factor. *SH5*, highly homologous to *qSH1*, also BEL1-type homeobox gene, involves in controlling seed shattering (Yoon et al., 2014). *SHATTERING ABORTION1* (*SHAT1*) also found to be involved in seed shattering in rice (Zhou et al., 2012).

In domesticated sorghum (*Sorghum bicolor*), similar to rice, shattering occurs due to the loss of abscission layer in between the joint of seed hull and pedicel. A single gene, *Shattering1* (*Sh1*) encodes a YABBY transcription factor, controlling seed shattering in sorghum (Lin et al., 2012). In a wild sorghum relative (*Sorghum propinquum*), seed shattering is conferred by the gene *SpWRKY* (Tang et al., 2013).

In domesticated wheat (*Triticum aestivum*), *Q* gene encodes a member of AP2-family transcription factor, confers the free-threshing character (the loss of tendency of the spike

shattering). The *Q* gene is involved in the seed shattering process in domesticated wheat (Simons et al., 2006; Zhang et al., 2011; Dong and Wang, 2015).

### 2.11. Breeding of *Brassica napus* for shatter tolerance

The conventional way to breed *B. napus* for higher level of shatter tolerance, using shatter tolerance species from the triangle of U, has been based on resynthesis of *Brassica napus* or interspecific hybridization. To overcome chromosomal imbalance and improve fertility, this method requires several cycles of breeding and selection (Hossain et al., 2012). Interspecific hybridization between *B. juncea* and *B. napus*, carried out by Prakash and Chopra (1990), was able to develop *B. napus* plants with complete non-dehiscent fruits. The seed fertility, however, was very poor (23%) which indicated significant chromosomal imbalance.

There have been a few studies that used a transgenic approach to improve shatter resistance. To produce shatter resistant *Brassica* siliquae, ectopic expression of the *Arabidopsis FRUITFULL* gene in *B. juncea* is sufficient and the genetic pathway of valve margin specification is conserved between *Arabidopsis* and *Brassica*. Transgenic fruit produced this way were completely shatter-tolerant and were too tough to thrash with combine harvester (Ostergaard et al., 2006).

An alternative way to identify variation in shattering tolerant among *B. napus* cultivars is the TILLING (targeting induced local lesions in genomes) approach. It identified the single base-pair allelic variation in a target gene, and identified the mutant in a target gene without genetic transformation is the major advantage of this approach. Down-regulation of the IND (indehiscent) gene which caused indehiscence in *B. napus* was identified by using this approach (Laga et al., 2011). However, the mechanical harvesting of siliquae was tough and similar to the transgenic *B. napus*.

### 2.12. Association mapping and shattering resistance in *Brassica* species

Association mapping (AM) based upon linkage disequilibrium is an important tool to identify and validate the genetic linkages between molecular markers and traits of interest. Association mapping allows screening a large number of alleles at one locus, performing high resolution mapping and saving time. It overcomes the limitations of the QTL analysis that utilizes bi-parental populations (Raman et al., 2011).

In *B. rapa*, *B. napus*, *B. juncea*, and *B. carinata* germplasm, natural variation for shatter resistance exists. To reduce yield loss under different environmental conditions, shatter resistance in *B. napus* germplasm is not sufficient while *B. carinata* is more resistant to silique shattering than the others (Raman et al., 2017). Raman et al. (2014) reported, in a doubled haploid (DH) population derived from BLN2762/Surpass400 and a diverse panel of 181 lines of *B. napus*, that several quantitative trait loci (QTL) on chromosomes A03, A09, A10, and C03 account for genetic variation in shatter resistance. Using genome-wide association analysis, Liu et al. (2016) reported six significant QTL for pod shattering resistance were located on chromosomes A01, A06, A07, A09, C02, and C05 in a diverse panel of 143 *B. napus* accessions, and bi-parental DH and intermated populations. Raman et al. (2017) identified five statistically significant genetic loci associated with pod shatter resistance in *B. carinata* on chromosomes B1, B3, B8, and C5.

At least one consistent locus on linkage group A09 in Australian and Chinese germplasm, which maps in the vicinity of ARR18 (AUXIN RESPONSIVE REGULATOR 18) and MADS-box gene, *SHATTERPROOF* (*BnShp1*), controls pod shatter resistance. Genes such as *SHATTERPROOF1* (*SHP1*); *SHATTERPROOF2* (*SHP2*); *FRUITFULL* (*FUL*); *INDEHISCENT* (*IND*); *ALCALTRAZ* (*ALC*); and *REPLUMLESS* (*RPL*), are involved in a complex regulatory

network and also control pod shatter resistance in *A. thaliana*, and other heterologous systems (Raman et al., 2017). Pod dehiscence process in canola and *Arabidopsis* are similar. Brassica and *Arabidopsis* share 86% DNA homology in protein coding regions. Shattering has been reduced by transgenic manipulation in *Arabidopsis* (Jaradat et al., 2014).

Mongkolporn et al. (2003) identified three RAPD markers (RAC-$3_{900}$, RX-$7_{1000}$ and SAC-$20_{1300}$) in an F2 population derived from Torch X DS17D by utilizing bulk segregant analysis (BSA). Markers RAC-$3_{900}$ and RX-$7_{1000}$ were linked to the sh1 and sh2 major gene for shattering resistance while marker SAC-$20_{1300}$ was linked with SH1 and SH2 (dominant alleles) for shatter susceptibility. The author did not indicate the chromosomal location of the loci associated with shatter resistance.

### 2.13. Genomic prediction (GP) in *Brassica napus*

Genomic prediction (GP) is a cost-effective approach which also reduces the breeding cycles, and helps plant breeders to incorporate quantitative traits in their breeding programs by allowing them to select best parent for crossing. GP uses the molecular markers which present throughout the whole genome for predicting the breeding value of a genotyped population. It develops a statistical model to predict the breeding value of an untested line by combining the genotypic data and phenotypic data of a training population. The reference population is both phenotyped and genotyped, while the selection candidates are genotyped only. To select the line, GP focuses on marker identification. Thus, plant and animal breeders rapidly adopted genomic prediction (Meuwissen et al., 2001; Hayes et al., 2009; Weigel et al., 2010; Asoro et al., 2013; Crossa et al., 2014; Li et al., 2021) in their breeding programs.

Availability of genotyping platforms and increased access to molecular markers makes the genomic prediction feasible for the breeders to incorporate this tool in their breeding program

for highly quantitative traits (Jannink et al., 2010). While identifying QTLs through linkage mapping have some limitations when it comes to detecting all possible genetic signals. On the other hand, the whole-genome prediction (WGP) has the ability to account for all possible genetic signals (major and minor QTLs) detected in the analysis, and thus, do not have significant statistical limits (Meuwissen et al., 2001).

Koscielny et al. (2020) conducted an experiment on a double-haploid *Brassica napus* population derived from parents 'PB36' and 'PB56'. The ability of the parents differs in seed setting at high temperature. They investigated the prospects of genomic prediction on a breeding population, using GBLUP and rrBLUP, for heat-stress tolerant in canola. The prediction accuracy of the nine traits that were evaluated in this experiment ranges from 0.14 to 0.66, where 0.14 was for trait yield and 0.66 was for 1000-seed weight. They also reported that within the stress treatment the prediction accuracy was higher than that of control treatment for seven traits out of the nine traits evaluated.

Fikere et al. (2020) conducted an experiment using 202 spring canola lines grown under rain-fed and irrigated conditions in Australia and evaluated genomic prediction accuracies using genomic best linear unbiased prediction (GBLUP) model in different diseases, agronomic, and seed quality traits. They reported genomic prediction accuracy ranges from 0.29 to 0.69, where 0.29 was for emergence count and 0.69 for seed yield.

Fikere et al. (2018) evaluated genomic prediction accuracy for blackleg disease on a panel of 532 spring and winter canola lines grown and phenotyped in blackleg disease nurseries. They reported genomic prediction accuracy was ranges from 0.30 to 0.69 for the spring set of canola and 0.19 to 0.71 within the winter set of canola used in this study using GBLUP model.

Roy et al. (2021) reported genomic prediction ability ranges from 0.41 to 0.64 for the four traits investigated for Sclerotinia stem rot resistance in canola, evaluated in a panel of 187 canola germplasm phenotyped in the field. Another study conducted by Roy et al. (2022), reported 0.45 to 0.68 genomic prediction ability for sclerotinia stem rot resistance in canola, evaluated in a panel of 337 canola germplasm phenotyped in the greenhouse.

### 2.14. Genome editing for pod shattering resistance in *Brassica napus*

Genome editing is a powerful tool for crop improvement which allows to eliminate the undesirable part of the genome via site-specific genome editing. Different sequence-specific nucleases (SSN), responsible for double-stranded DNA breaks (DSB), are available for gene editing. The commonly used gene/genome editing methods are clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9 (CRISPR/Cas9), transcription activator-like effector nucleases (TALENs), zinc-finger nucleases (ZFNs), and meganucleases (MNs) (Doudna and Charpentier 2014: Zaman et al., 2019 a).

For fruit dehiscence, *INDEHISCENT* (*IND*) and *ALCATRAZ* (*ALC*) gene homologues have previously been reported to play an essential role. However, for pod shattering resistance in canola, their functions are yet to be fully understood. Using the CRISPR/Cas9 technology, the functions of these two genes were investigated in rapeseed by Zhai et al. (2019). They reported the *BnIND* gene function as essential for pod shatter, additionally, at the same time highly conserved in *Brassica* species. On the other hand, the *BnALC* gene has limited ability for pod shatter resistance in *Brassica napus*. Partially redundant roles of the homoeologous copies of the *BnIND* gene, *BnA03.IND* and *BnA03.IND*, for pod shattering in canola was also reported by the authors where *BnA03.IND* showed higher contributions than *BnC03.IND*.

The *JAGGED* (*JAG*) gene in Arabidopsis previously suggested a key factor involved in the regulatory web of dehiscence fruit, and its role in pod shatter resistance in canola is not understood. Multiplex genome editing using CRISPR/Cas9 system was carried out by Zaman et al. (2019 b) on five homoeologs of the *JAG* gene: *BnJAG.A02*, *BnJAG.C02*, *BnJAG.C06*, *BnJAG.A07*, and *BnJAG.A08*. They reported a drastic effect in the development of the lateral organs which involve in pod shape and size, caused by knockout mutagenesis of all homoeologs. Significant changes in the pod dehiscence zone was observed due to the knockout mutagenesis in *BnJAG.A08-NUB* gene.

Pod shattering materials in canola can be derived by downregulating the expression of *SHP1/2* genes and activating the *FUL* genes' expression (Ferrándiz et al., 2000; Gu et al., 1998). Zaman et al. (2021) used CRISPR-Cas9 system to characterize the functions of *BnSHP1/BnSHP2* genes homoeologs in *Brassica napus*. Eight *SHP* homoeologs were targated by CRISPR-Cas9 system to evaluate their contribution in pod shattering resistance in canola. Among the eight homoeologs, six of them belongs to *BnSHP1*: *BnSHP1A09*, *BnSHP1C08*, *BnSHP1C09*, *BnSHP 1C06*, *BnSHP1C04-B*, and *BnSHP1A04*; while two of them belongs to *BnSHP2*: *BnSHP2A05* and *BnSHP2C04-A*. The result suggested that *BnSHP1A09* control lignin contents at dehiscence zone. Meanwhile, adjacent to valves and replum, reduced lignified layer and separation layer were exhibited by *BnSHP1A09/C04-B/A04* and *BnSHP2A05/C04-A*.

# CHAPTER 3. MATERIALS AND METHODS

## 3.1. Field experiment and plant materials

A total of 150 spring type *Brassica napus* accessions obtained from the U.S. National Plant Germplasm System and NDSU canola breeding program were used in this study. The experiment was planted in a randomized complete block design (RCBD) with three replications at two field locations in 2020. The locations were at North Dakota State University campus, Fargo, and the other at Osnabrock, North Dakota. In addition, an unreplicated trial, using the same population panel, was planted in the Agricultural Experiment Station at Carrington, ND in 2020. In 2021, the study was planted in a RCBD with three replications in three locations: Fargo, Osnabrock and Carrington, ND. However, due to a sudden devastating hailstorm, in August 2021, the experiment at Carrington was lost.

Additionally, due to the extreme drought weather condition, late flowering and poor/insufficient pods set the Fargo location in 2021 had to be discarded. Each germplasm was planted in single-rows plots. The row length was 2.0 m long. About 30 – 35 plants were grown per row. Regular intercultural practices were done to manage the field.

Before damage

After damage

Fig. 3.1. Damaged experimental locations (Carrington 2021) due to shattering caused by a sudden heavy hailstorm.

### 3.2. Greenhouse experiment and plant materials

The same 150 spring type *B. napus* accessions were used in the field study. The accessions were planted in the greenhouse in a RCBD with three replications at the Jack Dalrymple Agricultural Research Complex, NDSU. Each germplasm accession was planted in 4″ x 4″ pots, and four plants were grown per genotype and per replication. Greenhouse soil PRO

MIX BX general purpose was used to grow canola plants, and Osmocote® slow-release fertilizer (Scott's Company LLC, Marysville, OH, USA) was added into the soil mix to supply sufficient nutrients. The greenhouse temperature was maintained at $25^0$ C and 16 hours of photoperiod.

Fig. 3.2. Different stages of the experiment conducted in the greenhouse

### 3.3. Pod samples collection

At maturity, usually 85 to 110 days after planting, randomly selected 20 pods were collected from five plants per genotype per replication from each field location, and from four plants per genotype per replication from the greenhouse. Here, we referred the location-year combinations as environments, E1 (Osnabrock 2020), E2 (Fargo 2020), E3 (Carrington 2020), E4 (Osnabrock 2021) and E5 (Greenhouse 2021), and the pod samples were collected from 140, 129, 130, 95, and 135 genotypes, respectively.

### 3.4. Evaluation of pod shattering resistance

A total of 260 pods [240 pods (20 pods x 4 locations x 3 replications) + 20 pods (20 pods x 1 location x 1 replication)] pods were collected per genotype to evaluate the pod shatter resistance. The shattering resistance was measured by using a modification of the random impact test (RIT) method developed by Bruce et al. (2002). The collected pods were dried for two weeks at a constant temperature of 20 °C at 23% humidity in paper bags in the lab. Here, 20 intact pods per genotype per location were placed in a cylindrical container (17 cm height with slightly convex base diameter) with 15 steel balls (12.5 mm in diameter). The cylindrical container was placed in a horizontal shaker machine (Fig. 3.3). The shaker was running for 20 s with 350 rpm. The number of intact and shattered pods were counted and scored.

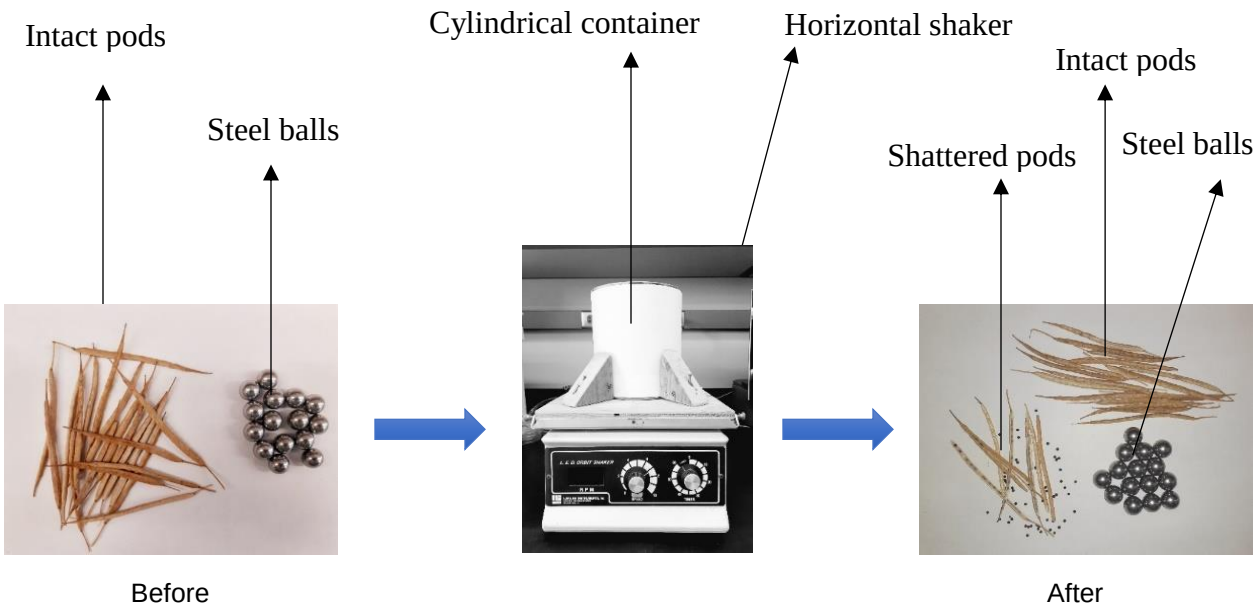


Fig. 3.3. Phenotypic assay to measure the pod shatter resistance

### 3.5. Genotyping

Young leaves from 30-day old plants were collected, frozen into liquid nitrogen, and stored at -80$^0$ c until DNA extraction. The lyophilized leaf tissues were ground and genomic DNA was extracted using Qiagen DNeasy Kit (Qiagen, CA, USA) following the manufacturer's protocol. DNA quantification was done using a NanoDrop 2000/2000c Spectrophotometer (Thermofisher Scientific). The *ApekI* enzyme was used to prepare the genotyping-by-sequencing (GBS) library where Elshire et al. (2011) protocol was followed. DNA sequencing was done using Illumina HiSeq 2500 sequencer at the University of Texas Southwestern Medical Center, Dallas, Texas, USA.

The GBS sequencing were aligned and SNP calls were conducted in the laboratory Oilseed Breeding Program, Department of Plant Sciences, North Dakota State University, Fargo, ND (Rahman et al. 2022). Briefly, the sequencing reads were aligned to the *Brassica napus* cultivar 'ZS 11' reference genome (Sun et al., 2017) using Bowtie 2 (version 2.3.0) alignment

tool (Langmead and Salzberg 2012). With the default parameter SNP calling was done using TASSEL 5 GBSv2 pipeline (Glaubitz et al., 2014). In total, 497,336 unfiltered SNPs were identified. VCFtools (Danecek et al., 2011) was used to filter and identify high-quality SNPs. These are the criteria that were considered to get the high-quality SNPs: minor allele frequency (MAF) ≥ 0.05, missing values (max-missing) ≤50%, depth (minDP) ≥ 5 and physical distance (thin) ≤ 500 bp. More than 25% SNPs that were heterozygous, considering canola is a self-pollinated crop, were removed using TASSEL (Bradbury et al., 2007). In addition, SNPs located outside of the chromosome (unknown position) were also removed (Rahman et al., 2022).

### 3.6. Statistical analysis

The pod shattering data were analyzed using SAS 9.4 (SAS Institute Inc., USA) individually by E1, E2, E4 and E5 locations. LS mean was obtained and the analysis of variance (ANOVA) was calculated considering genotypes as fixed effect and replications as random effect. As E3 was un-replicated, hence, the original pod shattering data from E3 was used for further analysis for this location. Broad-sense heritability ($H^2$) was calculated using the variance components with the following equation:

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_{ge}^2}{n} + \frac{\sigma_e^2}{nr}}$$

Where,

$H^2$ = broad-sense heritability

$\sigma^2_g$ = genotypic variance

$\sigma^2_e$ = residual error variance

$\sigma^2_{ge}$ = genotype by environment variance

$n$ = number of environments

$r$ = number of replications per environment

### 3.7. Marker-trait association

The GAPIT R package (version 3) in R 4.0.4 was used for marker-trait association analysis. A total of 8 GWAS models was implemented for the association mapping. Among them, 5 of the models were single locus analysis algorithm: General Linear Model (GLM), Mixed Linear Model (MLM), Compressed MLM (CMLM), Enriched CMLM (ECMLM), and Settlement of MLM Under Progressively Exclusive Relationship (SUPER); whereas 3 of them were multi-locus analysis: Multiple Loci Mixed Linear Model (MLMM), Fixed and random model Circulating Probability Unification (FarmCPU), and Bayesian-information and Linkage-disequilibrium Iteratively Nested Keyway (BLINK). Three Principal components (PCs) and the kinship matrix were included in the model in the GAPIT analysis to control population structure and individual relatedness.

After removing the SNPs with less than 5% minor allele frequency (MAF), 24540, 23478, 23965, 23243, and 24066 high quality SNPs was remained from a total of 34,261 SNPs for the environment E1, E2, E3, E4, and E5, respectively, for the GWAS analysis. Li and Ji (2005) was used to determine the significant threshold of *P* value for the SNPs and trait association. Among the respective high-quality SNPs in different environments, the effective number of independent tests (Meff) were obtained by calculating the correlation matrix and eigenvalue decomposition. Further, to get the significant *P* value, Bonferroni correction was applied on the effective number of independent tests where $\alpha = 0.5$. The effective number of independent tests were determined as 203, 200, 191, 203, and 196 in the environment E1, E2, E3, E4, and E5, respectively. Hence the significant threshold of *P* value for the environments are:

E1 is $P = 0.05/203 = 0.00025$ or $-\log(P) = 3.6$; equivalent to $P \leq 0.00025$ or $-\log_{10}(P) \geq 3.6$

E2 is $P = 0.05/200 = 0.00025$ or $-\log(P) = 3.6$; equivalent to $P \leq 0.00025$ or $-\log_{10}(P) \geq 3.6$

E3 is P = 0.05/191 = 0.00026 or -log (P) = 3.6; equivalent to $P \leq 0.00026$ or $-\log_{10}(P) \geq 3.6$

E4 is P = 0.05/138 = 0.00036 or -log (P) = 3.4; equivalent to $P \leq 0.00036$ or $-\log_{10}(P) \geq 3.4$

E5 is P = 0.05/196 = 0.00026 or -log (P) = 3.6; equivalent to $P \leq 0.00026$ or $-\log_{10}(P) \geq 3.6$

### 3.8. Candidate genes identification

The identified significant SNPs were used to search for the candidate genes. The *B. napus* cultivar 'ZS 11' reference genome (Sun et al., 2017) was used for the genes model. Genes considered as candidate genes that are present within the 50 kb upstream and downstream of the significant SNPs on the reference genome. The gene annotation was determined by blasting the protein sequences from the gene model against TAIR 10.

### 3.9. Genomic prediction (GP)

A total of 14 genomic prediction models were used in our study. The parametric models included genomic best linear unbiased prediction (GBLUP) (VanRaden, 2008; Habier et al., 2013); EGBLUP (Jiang and Reif, 2015); ridge regression best linear unbiased prediction (rrBLUP) (Meuwissen et al., 2001; Endelman, 2011); the least absolute shrinkage and selection operator (LASSO) (Tibshirani, 1996; Usai et al., 2009); the elastic net (EN) (Zou and Hastie, 2005); Bayesian ridge regression (BRR) (Gianola et al., 2003; Desta and Ortiz 2014); Bayesian LASSO (BL) (Park et al., 2008); BayesA (BA) (Habier et al., 2011; Meuwissen et al., 2001); BayesB (BB) (Habier et al., 2011; Meuwissen et al., 2001); and BayesC (BC) (Habier et al., 2011; Meuwissen et al., 2001). In addition, the following non-parametric models were evaluated: reproducing kernel Hilbert space regression (RKHS) (Gianola and Van Kaam, 2008; De Los Campos et al., 2010); multiple kernel MRKHS (Gianola and Van Kaam, 2008; De Los Campos et al., 2010); support vector machines (SVM) (González-Recio et al., 2014); and random forest (RF) (Breiman et al., 2001). These models were implemented in the BWGS (BreedWheat Genomic Selection) pipeline in R, described by (Charmet et al., 2020). The same marker sets that

were used for the GWAS analysis, were also used in the genomic prediction: 24540, 23478, 23965, 23243, and 24066 high quality SNPs for the environment E1, E2, E3, E4, and E5, respectively. To carry out the genomic prediction analysis in the BWGS pipeline in R, we used the high-performance computing (HPC) platform available at CCAST, NDSU. [Note: "This work used resources of the Center for Computationally Assisted Science and Technology (CCAST) at North Dakota State University, which were made possible in part by NSF MRI Award No. 2019077."]

Although the ridge regression (RR) was implemented as one of the 14 GP models in the integrated pipeline BWGS, we used the genomic prediction (GP) model ridge regression best linear unbiased prediction (rrBLUP) as reference model to compare the predictive ability of those 14 GP models implemented in the BWGS pipeline in R. We separately used the model rrBLUP in R as a reference model. The model was constructed using the 'rrBLUP' (Endelman, 2011) package in R. The constructed model is represented as:

$$y = \mu + X\beta + \varepsilon$$

Where,

$y$ = vector of the phenotypic observations,

$\mu$ = grand mean

$X$ = marker genotype matrix

$\beta$ = estimated random additive marker effect

$e$ = residual error term.

The genomic prediction ability of the GP model was assessed with a five-fold cross validation with 100 rounds of random sampling or 100 iteration or 100 replications.

As the true breeding value of the pod shattering is unknown, we calculate the prediction accuracy by the correlation between the genomic estimated breeding values and the observed phenotypic values divided by the square root of the phenotypic heritability ($\sqrt{H^2}$) (Jarquín et al., 2014; Roy et al., 2021; Roy et al., 2022). Thus, the prediction accuracy of each model for pod shattering was estimated by dividing the mean predictive ability by square root of heritability.

# CHAPTER 4. RESULTS

## 4.1. Phenotypic variation

Significant differences (P < 0.0001) were observed within the *B. napus* genotypes used in this study with respect to pod shatter resistance scores (Table 4.1). The shattering resistance scores ranged from 0.67 to 18.33 with mean 11.11 for environment one E1 (Osnabrock 2020) within 140 genotypes (Fig. 4.1.) , 0.33 to 16.0 with mean 6.26 for environment two E2 (Fargo 2020) within 129 genotypes (Fig. 4.2.), 0.00 to 19.00 with a mean 10.88 in environment three E3 (Carrington 2020) within 130 genotypes (Fig. 4.3.), 0.00 to 18.67 with a mean 8.62 in environment four (Osnabrock 2021) within 95 genotypes (Fig. 4.4.), 0.33 to 17.00 with mean 8.33 in environment five E5 (Greenhouse 2021) within 134 genotypes (Fig. 4.5.). The heritability of the pod shatter resistance ranges from 74 % to 87 % across the environments where 74 % in E1, 87 % in E2, 86 % in E4, and 86 % in E5 (Appendix: Table A.1.). The genotype's details are in the Appendix: Table A.1.

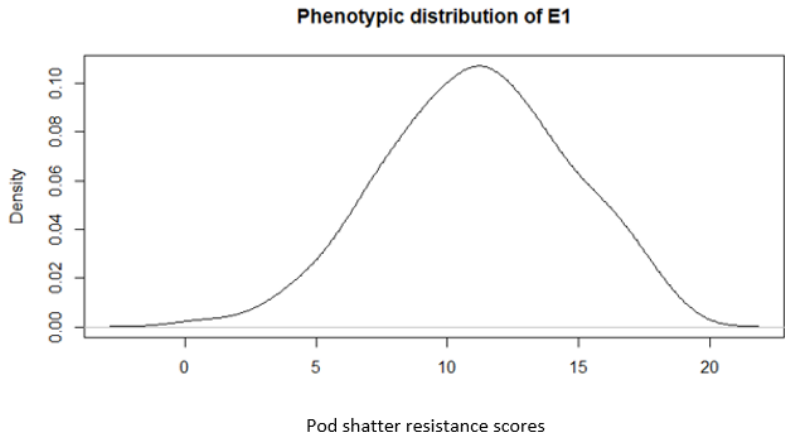


Fig. 4.1. Phenotypic distribution of the canola pod shattering score data in environment E1 (Osnabrock 2020)

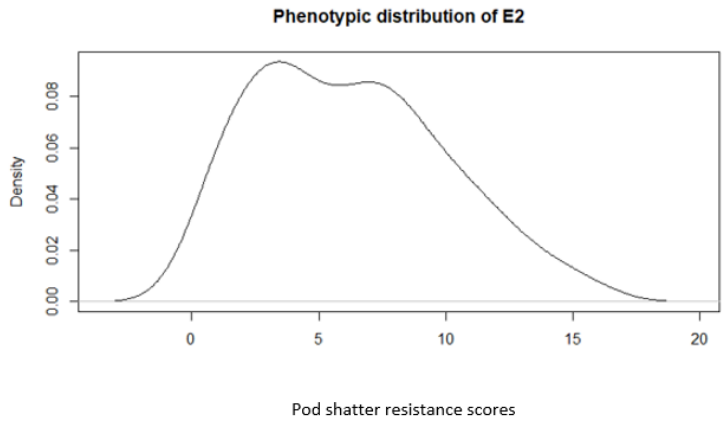


Fig. 4.2. Phenotypic distribution of the canola pod shattering score data in environment E2 (Fargo 2020)

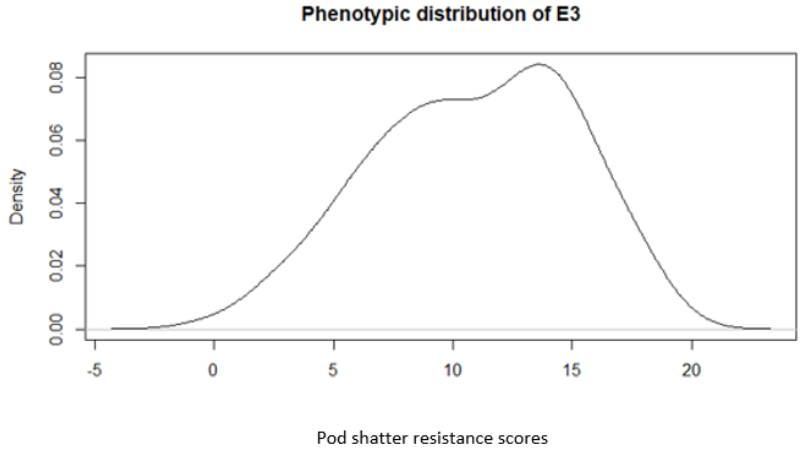


Fig. 4.3. Phenotypic distribution of the canola pod shattering score data in environment E3 (Carrington 2020)

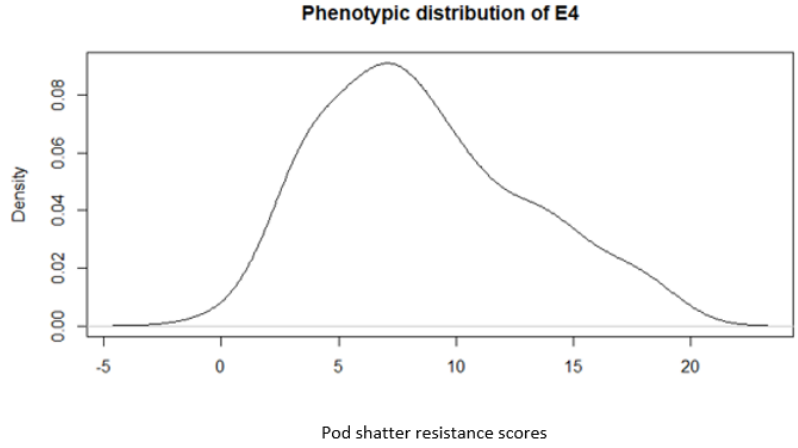


Fig. 4.4. Phenotypic distribution of the canola pod shattering score data in environment E4 (Osnabrock 2021)

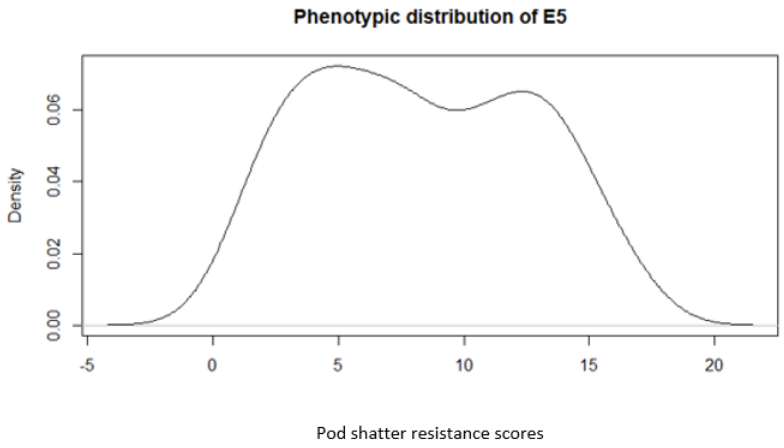


Fig. 4.5. Phenotypic distribution of the canola pod shattering score data in environment E5 (Greenhouse 2021)

Table 4.1. Pod shattering resistant scores (LS mean) on a scale of 20, where 20 being the highly resistant and 0 being highly susceptible to pod shattering in *B. napus* genotypes used in this study among environments.

| Genotypes | Name | Environments* | | | | |
|---|---|---|---|---|---|---|
| | | E1 | E2 | E3 | E4 | E5 |
| N_16 | Aviso | 7.3 | . | 6.0 | 5.0 | 6.3 |
| N_26 | Bingo | 9.3 | . | 14.0 | . | . |
| N_28 | BO-63 | 11.3 | 1.3 | 8.0 | 7.0 | 5.0 |
| N_33 | Brio | 11.0 | 5.0 | 11.0 | 4.0 | 3.3 |
| N_34 | Bronowski | 11.3 | 5.0 | 7.0 | 7.3 | 2.0 |
| N_35 | Buk Wuk 3 | 5.7 | 1.3 | 4.0 | . | 2.7 |
| N_41 | Ceskia Tabor | 10.0 | 1.0 | 5.0 | . | 6.7 |
| N_46 | Colt | . | 11.3 | 7.0 | . | . |
| N_49 | Comet | 16.7 | 4.3 | 18.0 | 6.3 | 12.3 |
| N_50 | Conquest | 9.3 | 6.7 | 17.0 | 12.3 | 13.7 |
| N_52 | Cougar | 9.0 | 3.7 | 14.0 | 6.3 | 4.7 |
| N_55 | Cresor | 11.3 | 2.0 | 10.0 | . | 7.7 |
| N_56 | Cresus | 14.0 | . | . | . | 5.3 |
| N_58 | Crop | . | . | 13.0 | . | 11.3 |
| N_61 | Czyzowski | 9.3 | 4.7 | 9.0 | . | 14.0 |
| N_65 | Delta | 12.3 | 6.7 | 12.0 | 4.0 | 3.3 |
| N_72 | Drakkar | 8.0 | 5.7 | 13.0 | . | 4.0 |
| N_86 | Evvin | 12.0 | 6.3 | 9.0 | 8.0 | 2.3 |
| N_91 | Flint | 16.0 | 4.0 | 13.0 | . | 14.3 |
| N_92 | Fonto | 8.7 | 3.7 | 6.0 | 6.3 | 3.7 |
| N_93 | France 1 | 14.0 | . | . | 10.7 | 7.3 |
| N_98 | Fuji | 3.0 | . | . | . | . |
| N_100 | Galant | 12.3 | 1.7 | 10.0 | . | 8.0 |
| N_101 | Galaxy | 16.0 | 16.0 | 17.0 | . | 16.0 |
| N_104 | Gido | 11.3 | 3.0 | 12.0 | . | 7.3 |
| N_106 | Gisora | . | . | . | . | 13.7 |
| N_109 | Global | 7.0 | 3.0 | . | 7.0 | 5.0 |
| N_110 | Golden | 12.7 | 3.0 | 16.0 | . | 8.3 |
| N_112 | Gora | 10.7 | 13.7 | 19.0 | . | 13.7 |
| N_115 | Gulle | 7.0 | 1.0 | 8.0 | . | 4.3 |
| N_116 | Gullivar | 11.0 | 2.7 | 8.0 | . | 5.0 |
| N_119 | Hi-Q | 16.0 | 9.3 | 15.0 | 6.0 | 7.7 |
| N_124 | INRA-R-2000 | 10.7 | 6.7 | . | . | 8.7 |
| N_125 | IR-2 | 10.7 | 7.7 | 14.0 | 8.0 | 10.7 |
| N_130 | Janetzkis | 16.7 | 7.7 | 14.0 | 9.3 | 9.7 |
| N_133 | Jasna | 12.0 | 8.3 | 12.0 | . | 13.3 |
| N_136 | Kanada | 14.3 | 8.0 | 14.0 | 9.3 | 13.0 |
| N_140 | Klinki | 12.3 | 12.0 | 15.0 | . | 14.0 |
| N_142 | Kosa | 11.0 | . | 6.0 | . | 4.7 |
| N_143 | Koubun | . | . | . | . | 0.3 |
| N_144 | Kovalevskjj | 9.3 | 5.3 | 8.0 | 6.0 | 3.0 |
| N_145 | Kraphhauser | 17.7 | 7.0 | 17.0 | . | 12.0 |
| N_152 | Laura | 14.3 | . | . | . | . |
| N_154 | Legend | 6.0 | 0.3 | 5.0 | 2.3 | 1.3 |
| N_160 | Lifura | 9.0 | 3.7 | 9.0 | . | 2.3 |
| N_183 | Mar'janovskij | 4.3 | 1.0 | 2.0 | 3.3 | 1.7 |
| N_185 | Mazowiecki | 9.7 | 4.3 | 9.0 | . | 6.3 |
| N_187 | Midas | 7.3 | 0.7 | 10.0 | . | 2.3 |
| N_188 | Miekuro Dane | 7.7 | . | 4.0 | . | 2.7 |

Table 4.1. Pod shattering resistant scores (LS mean) on a scale of 20, where 20 being the highly resistant and 0 being highly susceptible to pod shattering in *B. napus* genotypes used in this study among environments (continued).

| Genotypes | Name | Environments* | | | | |
|---|---|---|---|---|---|---|
| | | E1 | E2 | E3 | E4 | E5 |
| N_197 | Mura yamasho | 8.3 | 6.7 | 10.0 | . | 7.0 |
| N_202 | NDC-A14026 | 11.3 | 10.0 | 8.0 | 4.7 | 4.3 |
| N_203 | NDC-A14032 | 13.3 | 8.7 | 15.0 | . | 13.3 |
| N_204 | NDC-A14033 | 9.3 | 6.7 | 11.0 | . | 13.3 |
| N_205 | NDC-A14035 | 5.3 | 2.7 | 10.0 | 4.3 | 4.0 |
| N_206 | NDC-A14036 | 6.7 | 0.7 | 8.0 | 3.7 | 6.3 |
| N_207 | NDC-A14045 | 18.0 | 8.3 | 16.0 | . | 14.0 |
| N_208 | NDC-A14046 | 8.3 | 5.7 | 10.0 | 3.7 | 10.7 |
| N_209 | NDC-A14050 | 16.0 | 11.7 | 18.0 | . | 13.7 |
| N_210 | NDC-A14055 | 13.3 | 12.7 | 16.0 | . | 12.3 |
| N_211 | NDC-A14056 | 11.0 | 5.0 | 13.0 | 2.3 | 5.7 |
| N_212 | NDC-E12009 | 15.7 | 9.3 | 18.0 | 12.3 | 13.3 |
| N_213 | NDC-E12023 | 13.3 | 5.7 | 13.0 | 13.7 | 5.7 |
| N_214 | NDC-E12025 | 10.7 | 5.0 | 8.0 | 6.7 | 10.3 |
| N_215 | NDC-E12027 | 12.3 | 11.0 | 14.0 | 16.7 | 13.3 |
| N_216 | NDC-E12044 | 16.3 | 12.0 | 14.0 | 12.0 | 13.0 |
| N_217 | NDC-E12079 | 13.7 | 10.7 | 10.0 | . | 11.7 |
| N_218 | NDC-E12081 | 12.7 | 9.7 | 13.0 | 7.3 | 14.7 |
| N_219 | NDC-E12086 | 14.3 | 8.3 | . | 0.0 | 15.3 |
| N_220 | NDC-E12119 | 9.0 | 7.7 | 8.0 | 5.7 | 6.7 |
| N_221 | NDC-E12120 | 14.0 | 6.7 | 17.0 | 12.0 | 16.7 |
| N_222 | NDC-E12121 | 13.3 | 7.7 | 18.0 | . | 8.3 |
| N_223 | NDC-E12131 | 9.0 | 8.0 | 13.0 | 11.0 | 15.7 |
| N_224 | NDC-E12133 | 7.0 | 6.7 | . | 8.7 | 9.7 |
| N_225 | NDC-E13193 | 9.0 | 9.7 | . | 7.7 | 8.0 |
| N_226 | NDC-E13279 | 11.0 | 5.0 | . | 6.0 | 6.0 |
| N_227 | NDC-E13285 | 12.7 | 8.7 | 12.0 | 7.7 | 12.3 |
| N_228 | NDC-E15031 | 13.0 | 9.0 | 15.0 | . | 12.3 |
| N_229 | NDC-E15146 | 12.3 | 14.0 | 15.0 | 10.3 | 8.0 |
| N_230 | NDC-E15174 | 7.3 | 1.7 | 9.0 | 3.3 | 1.7 |
| N_231 | NDC-E15200 | 14.0 | 8.0 | 17.0 | . | 15.3 |
| N_232 | NDC-E15234 | 10.3 | 6.7 | . | 4.3 | 14.7 |
| N_233 | NDC-E15294 | 6.7 | 8.7 | 5.0 | 6.0 | 6.0 |
| N_234 | NDC-E16015 | 13.0 | 15.0 | 16.0 | 3.3 | 15.0 |
| N_235 | NDC-E16053 | 5.0 | 2.0 | 6.0 | 8.3 | 5.0 |
| N_236 | NDC-E16152 | 15.3 | 11.0 | 15.0 | 13.0 | 16.3 |
| N_237 | NDC-E16169 | 11.7 | 5.0 | 12.0 | 7.3 | 11.3 |
| N_238 | NDC-E16198 | 16.7 | 13.7 | 15.0 | 14.3 | 12.0 |
| N_239 | NDC-E17132 | 15.0 | . | 17.0 | . | 10.3 |
| N_240 | NDSU01104 | 9.0 | 3.3 | 8.0 | 10.0 | 5.0 |
| N_241 | NDSU0417 | 14.3 | 6.7 | 7.0 | 7.0 | 2.0 |
| N_242 | NDSU0472 | 15.3 | 6.3 | 15.0 | . | 4.0 |
| N_243 | NDSU0473 | 13.7 | 3.7 | 15.0 | 11.7 | 11.7 |
| N_244 | NDSU0474 | 17.3 | 7.3 | 10.0 | 10.0 | 10.7 |
| N_245 | NDSU0475 | 17.3 | 7.7 | 14.0 | 10.3 | 10.0 |
| N_246 | NDSU0521 | 11.3 | 4.0 | 5.0 | 8.7 | 11.0 |
| N_247 | NDSU0522 | 9.7 | 10.0 | 11.0 | 9.0 | 10.7 |
| N_248 | NDSU0619 | 16.3 | 10.7 | 14.0 | 8.0 | 5.3 |
| N_249 | NDSU0620 | 14.0 | 7.7 | 11.0 | 10.3 | 12.0 |

Table 4.1. Pod shattering resistant scores (LS mean) on a scale of 20, where 20 being the highly resistant and 0 being highly susceptible to pod shattering in *B. napus* genotypes used in this study among environments (continued).

| Genotypes | Name | Environments* | | | | |
|---|---|---|---|---|---|---|
| | | E1 | E2 | E3 | E4 | E5 |
| N_250 | NDSU0726 | 8.0 | 3.3 | . | 8.0 | 4.7 |
| N_251 | NDSU0728 | 10.3 | 1.7 | . | 17.3 | 1.7 |
| N_252 | NDSU0729 | 11.7 | 7.3 | 11.0 | 10.7 | 8.7 |
| N_253 | NDSU10999 | 12.7 | 8.3 | 15.0 | 16.7 | 17.0 |
| N_254 | NDSU12989 | 12.0 | 12.7 | 14.0 | 7.7 | 10.7 |
| N_255 | NDSU151000 | 15.3 | 15.0 | 13.0 | 18.7 | 16.7 |
| N_256 | NDSU15989 | 13.7 | 8.7 | 15.0 | 18.3 | 13.7 |
| N_257 | NDSU161013 | 11.3 | 12.0 | 7.0 | 14.0 | 11.3 |
| N_258 | NDSU31001 | 7.3 | 5.3 | 10.0 | 7.7 | . |
| N_259 | NDSU31011 | 10.3 | 4.0 | 12.0 | 14.3 | 6.0 |
| N_260 | NDSU41000 | 18.0 | 3.7 | 15.0 | 14.3 | 14.7 |
| N_261 | NDSU7997 | 18.3 | 10.3 | 17.0 | 10.3 | 11.3 |
| N_262 | NDSU81000 | 12.7 | 11.3 | 12.0 | 8.7 | 7.3 |
| N_263 | NDSU91013 | 10.7 | 2.7 | 6.0 | 14.0 | 7.3 |
| N_270 | NU 41737 | 7.3 | 0.7 | 0.0 | 3.3 | 1.0 |
| N_271 | NU 51084 | 9.7 | 2.3 | 13.0 | 6.0 | 8.0 |
| N_280 | Oro | 0.7 | . | 2.0 | 3.7 | 1.7 |
| N_281 | Orpal | 12.7 | . | 14.0 | . | . |
| N_283 | Peace | 15.3 | 6.0 | 9.0 | 14.0 | 13.0 |
| N_288 | Polo canola | 7.0 | 1.7 | 3.0 | 4.3 | 3.0 |
| N_289 | Premier | 11.0 | 2.0 | 9.0 | 3.7 | 4.3 |
| N_291 | Printol | 8.7 | 5.0 | 9.0 | 10.7 | 9.0 |
| N_292 | Prota | 9.3 | 1.0 | 5.0 | 13.7 | 1.0 |
| N_293 | Q2 | 12.7 | 6.0 | 11.0 | 18.7 | 6.7 |
| N_302 | Ratnik | 8.7 | 3.0 | 13.0 | 6.3 | 9.3 |
| N_305 | Regent | 15.0 | 0.7 | 9.0 | 15.3 | 8.0 |
| N_306 | Regina II | 8.3 | 3.3 | 10.0 | 9.7 | 7.3 |
| N_307 | Reston | 4.7 | 1.7 | 5.0 | 17.0 | 4.3 |
| N_308 | Rico | 6.7 | 3.7 | 6.0 | . | 3.0 |
| N_311 | Romeo | 13.3 | 9.0 | 7.0 | 7.0 | 3.3 |
| N_315 | Russia 5 | 5.3 | 2.7 | 9.0 | 13.3 | 2.7 |
| N_317 | S.V. Gulle | 3.3 | . | 7.0 | . | . |
| N_322 | Seoul | 11.3 | 3.0 | 13.0 | 5.7 | . |
| N_327 | Silex | 6.7 | 0.7 | 3.0 | 3.0 | 2.3 |
| N_334 | Sunrise | 11.3 | 3.0 | 11.0 | 16.0 | 7.3 |
| N_335 | Sval of Gullen | 4.7 | 6.7 | 6.0 | 2.7 | 4.7 |
| N_340 | Taiwan | 7.3 | 10.0 | 14.0 | . | . |
| N_345 | Tanto | . | 4.0 | 3.0 | . | . |
| N_346 | Target | . | . | 3.0 | . | . |
| N_348 | Tobin | 10.3 | . | . | . | . |
| N_351 | Tonus | 9.7 | 4.0 | 7.0 | 4.0 | 3.3 |
| N_352 | Topas | 9.0 | 2.7 | 15.0 | . | 9.3 |
| N_354 | Tower | 9.3 | 12.7 | 7.0 | 2.7 | 6.7 |
| N_359 | Turret | 14.0 | 10.0 | 12.0 | 8.7 | 9.7 |
| N_366 | Vostochno-sibirskii | 10.3 | 4.0 | 10.0 | 5.0 | 10.3 |
| N_367 | Wasefuji | 11.3 | 8.0 | 14.0 | 5.7 | 4.3 |
| N_370 | Westar | 11.7 | 2.7 | 12.0 | 8.3 | 8.7 |
| N_374 | Willa | 16.0 | 10.3 | 12.0 | . | 12.3 |

* E1 = Osnabrock 2020, E2 = Fargo 2020, E3 = Carrington 2020, E4 = Osnabrock 2021, and E5 = Greenhouse 2021.

### 4.2. Marker trait association

Eight GWAS models, GLM, MLM, CMLM, ECMLM, SUPER, MLMM, FarmCPU, and BLINK, were implemented for the marker trait association analysis. The significant SNPs were selected though the following process. First, the SNPs that passed the significant *P*-threshold value in each respective environment were selected. Second, within the selected SNPs, the SNPs that were present in more than one environment or / and the SNPs that were detected by more than two GWAS models implemented within the environment were considered as significant / stable SNPs. The significant *P*-threshold value was determined as $-\log_{10}(P) \geq 3.6$ for E1, E2, E3, and E5, and $-\log_{10}(P) \geq 3.4$ for E4. The SNPs that passes the above-mentioned criteria were reported here and considered as stable or significant SNPs associated with pod shatter resistant in canola.

A total of 21 significant SNPs were identified (Table 4.2) associated with the pod shatter resistance in *B. napus*. These SNPs explained 3.8 – 25.4 % phenotypic variation. One SNP on chromosome A05, A10, C12, C15, C18, and C19, two SNPs on chromosome A01, A03, and A09, and three SNPs on chromosome A06, C13, and C14 were detected for pod shatter resistance (Fig. 4.6.). The Manhattan plot and Q-Q plot are showing the association between markers and traits in different environments (Fig. 4.7 to Fig. 4.11).

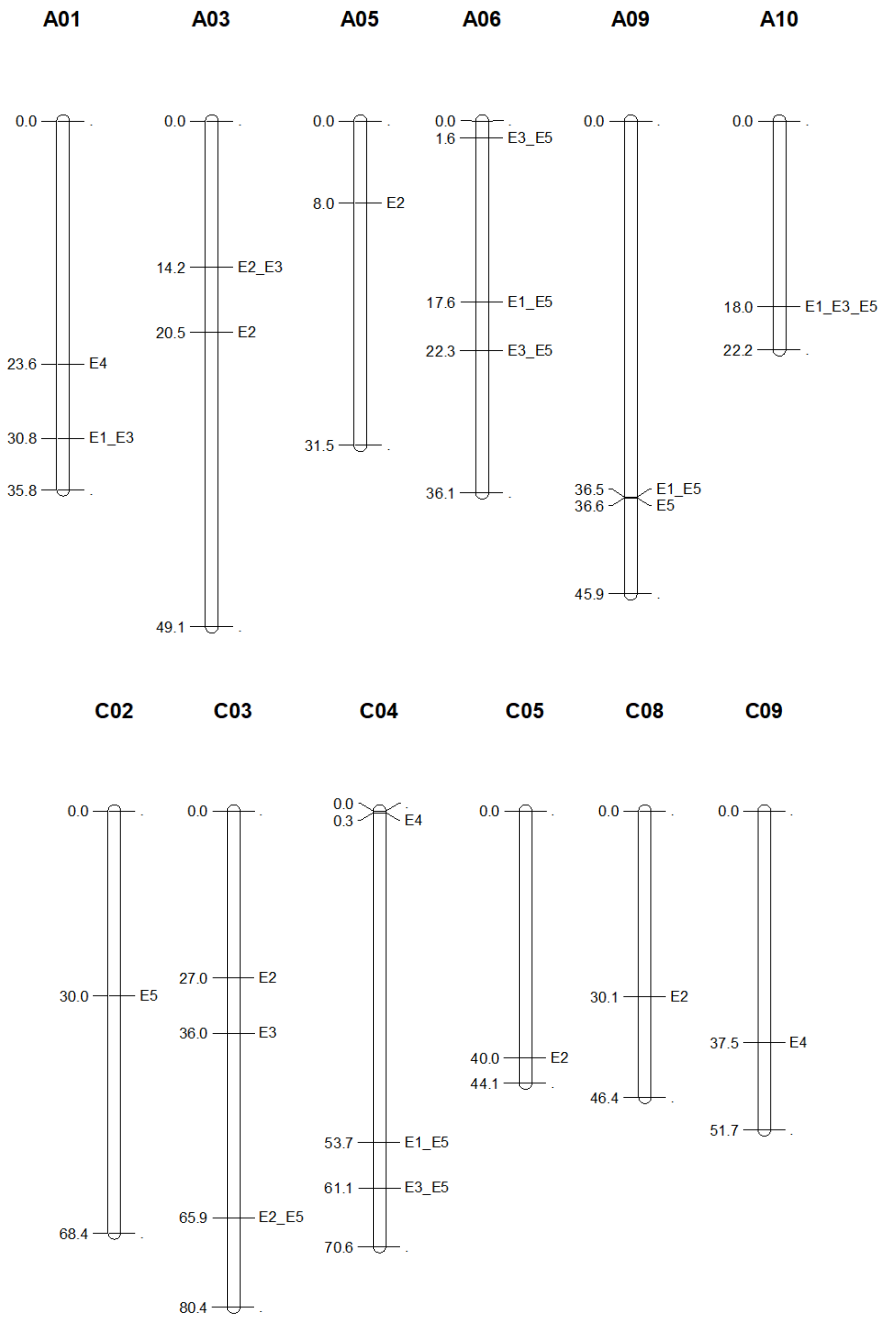


Fig. 4.6. Significant SNPs for pod shattering across the chromosomes of *B. napus*. Note: E1 = Osnabrock 2020, E2 = Fargo 2020, E3 = Carrington 2020, E4 = Osnabrock 2021, and E5 = Greenhouse 2021.

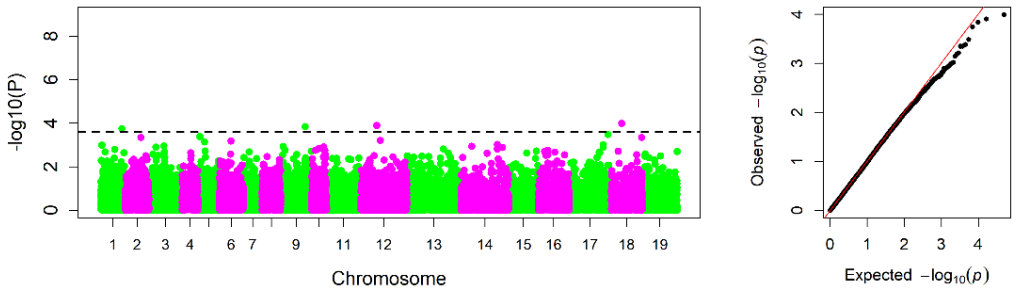


Fig. 4.7. Manhattan and Q-Q plots from FarmCPU model showing marker trait association for pod shatter resistance in canola in E1 (Osnabrock 2020)

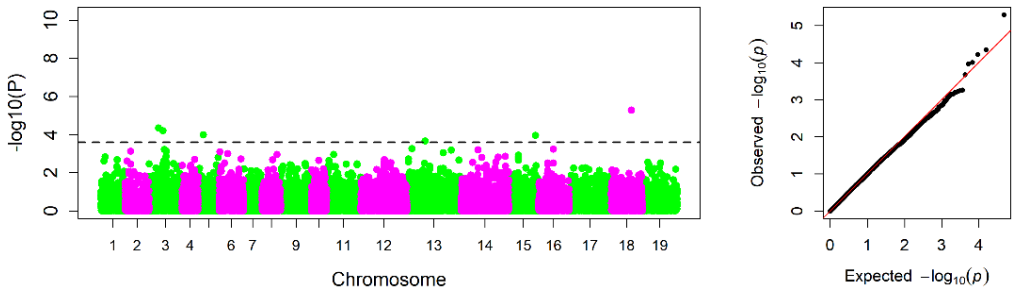


Fig. 4.8. Manhattan and Q-Q plots from FarmCPU model showing marker trait association for pod shatter resistance in canola in E2 (Fargo 2020)

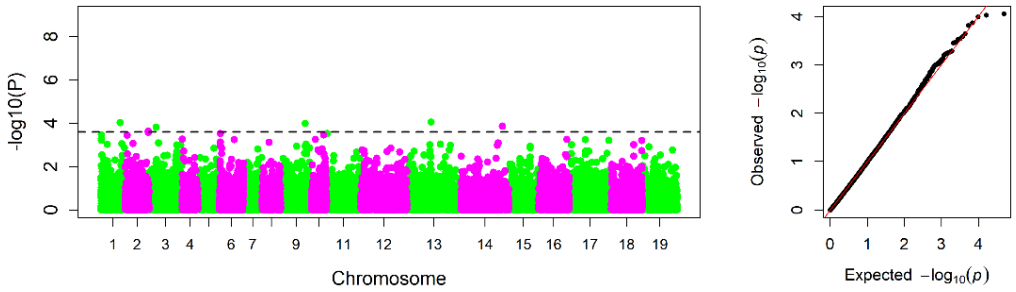


Fig. 4.9. Manhattan and Q-Q plots from FarmCPU model showing marker trait association for pod shatter resistance in canola in E3 (Carrington 2020)

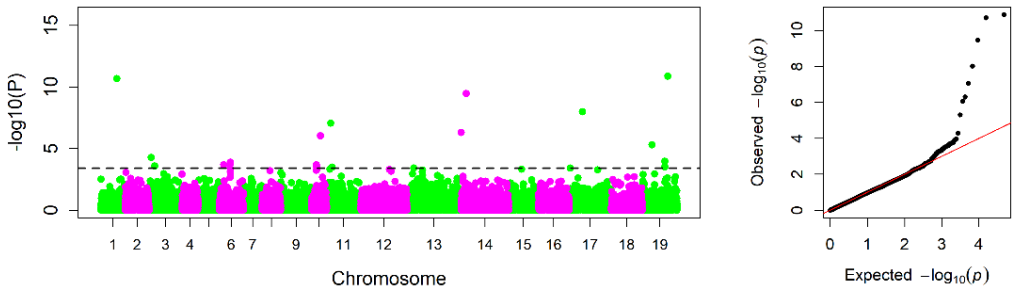


Fig. 4.10. Manhattan and Q-Q plots from FarmCPU model showing marker trait association for pod shatter resistance in canola in E4 (Osnabrock 2021)

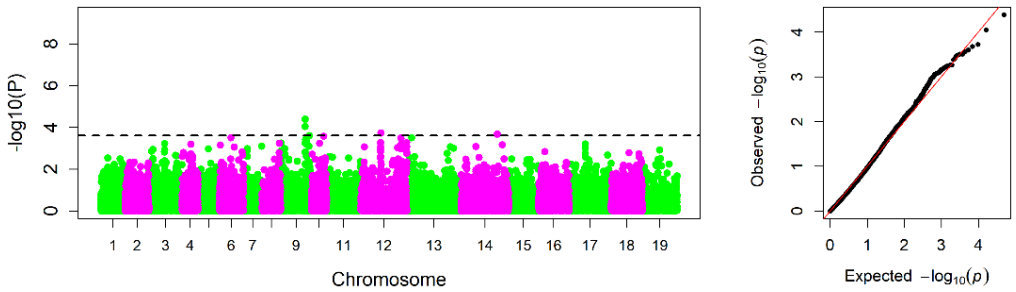


Fig. 4.11. Manhattan and Q-Q plots from FarmCPU model showing marker trait association in for pod shatter resistance in canola E5 (Greenhouse 2021)

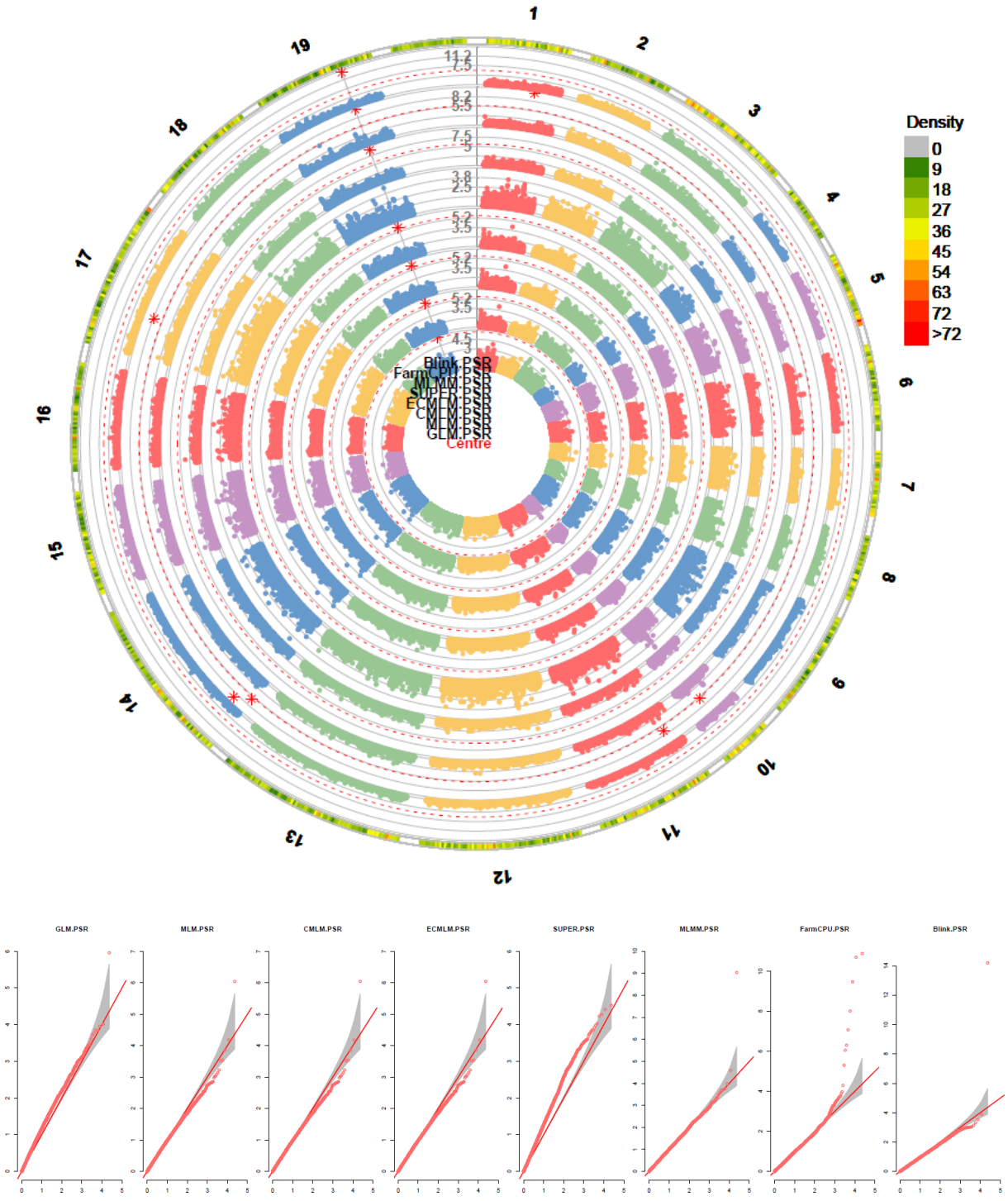


Fig. 4.12. Circular Manhattan and multiple Q-Q plots showing marker trait association for pod shatter resistance in canola, from all GWAS models implemented in E4 (Osnabrock 2021). Note: the number 1 – 19 on the circular Manhattan plot refer to the 19 chromosome of *B. napus*.

Table 4.2. List of Significant SNPs associated with pod shatter resistance in *Brassica napus* evaluated in five environments

| Sl. No. | SNP | Chr. | Position | -log10 (P) | maf | $R^2$ (%) | GWAS Model | Env.* |
|---|---|---|---|---|---|---|---|---|
| 1 | SCM002759.2_23551099 | 1 | 23551099 | 10.7 | 0.07 | 15.9 | GLM, MLM, CMLM, ECMLM, & FarmCPU | E4 |
| 2 | SCM002759.2_30842669 | 1 | 30842669 | 3.74 | 0.07 | 7.5 | GLM, FarmCPU, & BLINK | E1, E3 |
| 3 | SCM002761.2_20508257 | 3 | 20508257 | 4.22 | 0.34 | 11.2 | GLM, MLM, SUPER, & FarmCPU | E2 |
| 4 | SCM002761.2_14185096 | 3 | 14185096 | 4.34 | 0.46 | 8.3 | GLM, MLM, SUPER, & FarmCPU | E2, E3 |
| 5 | SCM002763.2_8035850 | 5 | 8035850 | 4 | 0.15 | 9.8 | GLM, MLM, FarmCPU & BLINK | E2 |
| 6 | SCM002764.2_1627549 | 6 | 1627549 | 4 | 0.29 | 5.7 | GLM, FarmCPU | E5, E3 |
| 7 | SCM002764.2_17583510 | 6 | 17583510 | 4.3 | 0.27 | 5.9 | GLM & FarmCPU | E5, E1 |
| 8 | SCM002764.2_22318710 | 6 | 22318710 | 3.8 | 0.05 | 4.5 | GLM, SUPER, & FarmCPU | E5, E3 |
| 9 | SCM002767.2_36584139 | 9 | 36584139 | 4.04 | 0.38 | 7.6 | GLM, SUPER, MLM, MLMM, & FarmCPU | E5 |
| 10 | SCM002767.2_36571589 | 9 | 36571589 | 4.38 | 0.46 | 6.9 | GLM, SUPER, MLM, FarmCPU,& BLINK | E5, E1 |
| 11 | SCM002768.2_18045825 | 10 | 18045825 | 4.2 | 0.07 | 3.8 | GLM & FarmCPU | E5, E1, E3 |
| 12 | SCM002770.2_29984333 | 12 | 29984333 | 3.72 | 0.22 | 8.5 | GLM, MLM, CMLM, ECMLM, SUPER, MLMM, & FarmCPU | E5 |
| 13 | SCM002771.2_27016886 | 13 | 27016886 | 3.68 | 0.17 | 10.2 | GLM, MLM, SUPER, MLMM, & FarmCPU | E2 |

Table 4.2. List of Significant SNPs associated with pod shatter resistance in *Brassica napus* evaluated in five environments (continued)

| Sl. No. | SNP | Chr. | Position | -log10 (P) | maf | $R^2$ (%) | GWAS Model | Env.* |
|---|---|---|---|---|---|---|---|---|
| 14 | SCM002771.2_35971759 | 13 | 35971759 | 4.05 | 0.19 | 9.8 | GLM, MLM, CMLM, ECMLM, MLMM, FarmCPU, & BLINK | E3 |
| 15 | SCM002771.2_65850033 | 13 | 65850033 | 3.4 | 0.43 | 8.7 | FarmCPU & MLM | E5, E2 |
| 16 | SCM002772.2_332092 | 14 | 332092 | 6.3 | 0.09 | 12.96 | GLM, MLM, CMLM, ECMLM, & FarmCPU | E4 |
| 17 | SCM002772.2_53679680 | 14 | 53679680 | 3.68 | 0.07 | 5.8 | GLM & FarmCPU | E5, E1 |
| 18 | SCM002772.2_61124720 | 14 | 61124720 | 3.17 | 0.06 | 7.8 | GLM, FarmCPU, & BLINK | E5, E3 |
| 19 | SCM002773.2_39972323 | 15 | 39972323 | 3.97 | 0.25 | 9.7 | GLM, MLM, MLMM, FarmCPU, & BLINK | E2 |
| 20 | SCM002776.2_30124447 | 18 | 30124447 | 5.29 | 0.07 | 11.3 | GLM, MLM, CMLM, ECMLM, SUPER, MLMM, FarmCPU, & BLINK | E2 |
| 21 | SCM002777.2_37526019 | 19 | 37526019 | 10.89 | 0.08 | 25.4 | GLM, MLM, CMLM, ECMLM, SUPER, MLMM, FarmCPU, & BLINK | E4 |

* E1 = Osnabrock 2020, E2 = Fargo 2020, E3 = Carrington 2020, E4 = Osnabrock 2021, and E5 = Greenhouse 2021.

## 4.3. Candidate genes

The 21 significant SNPs were used to search for the candidate genes for pod shatter resistant in canola. Genes that are present within the 50 kb upstream and downstream of the significant SNPs with known function associated with pod shatter resistance are considered as candidate genes. Three genes (Table 4.3.) were identified as candidate genes involved in pod

shattering in canola, *IND* (*INDEHISCENT*), *AGL65*, and *MAN7*. These candidate genes are involved in fruit dehiscence, fruit valve development, plant-type cell wall loosening, seed germination, pollen development, polar nucleus fusion, regulation of transcription, DNA-templated, and others. The biological functions of these genes, gene annotations, and related information are listed in Table 4.3.

Table 4.3. Candidate genes for pod shatter resistance in *Brassica napus* within the 50 kb upstream and downstream of the significant SNPs

| SNP | Chr. | SNP position (kb) | Start and end position of the gene (kb) | Gene symbol and ID | Gene Annotation/Gene Description | Symbol (TAIR) | GO biological Function (TAIR) |
|---|---|---|---|---|---|---|---|
| SCM002761.2_20508257 | 3 (A03) | 20508257 | 20,557,135 - 20,557,893 | LOC106444851 | transcription factor IND-like | *IND* | polar nucleus fusion, regulation of transcription, DNA-templated |
| SCM002771.2_65850033 | 13 (C03) | 65850033 | 65,795,589 - 65,804,404 | LOC106362782 | agamous-like MADS-box protein AGL65 | *AGL65* | pollen development, pollen maturation, regulation of pollen tube growth, regulation of transcription by RNA polymerase II |
| SCM002772.2_53679680 | 14 (C03) | 53679680 | 61,140,526 - 61,142,683 | LOC106418079 | mannan endo-1,4-beta-mannosidase 3-like | *MAN7* | fruit dehiscence, fruit valve development, plant-type cell wall loosening, seed germination, organic substance metabolic process. |

### 4.4. Genomic prediction

A total of 14 genomic prediction (GP) models were tested with a five-fold cross validation with 100 iterations. The GP model SVM performed the poorest and produced negative value across all environments. The SVM value, across environments, ranges from – 0.19 to – 0.16. The other 13 GP model's performance remain relatively constant within the environments. Overall, all the GP model's performance, except SVM, were highest within the E5 environment and lowest within the E1 environment. The genomic prediction within each environment ranges from 0.18 – 0.35 in E1 (Fig. 4.13.), 0.33 – 0.38 in E2 (Fig. 4.14.), 0.23 – 0.38 in E3 (Fig. 4.15.), 0.34 – 0.38 in E4 (Fig. 4.16.), and 0.40 – 0.50 in E5 (Fig. 4.17.). RF gave the highest predictive ability of 0.50, while LASSO gave the lowest of 0.18. The prediction accuracy of GP models for pod shattering ranges from 0.21 – 0.54 across environments (Table 4.4.) where highest was in the environment E5 and lowest was in the Environment E1. All the model performed better in the environment E5 over others.

The genomic prediction ability of the reference rrBLUP model ranges from 0.36 - 0.50 across five environments. The predictive ability of the model for pod shattering in the environment E1, E2, E3, E4, and E5 were 0.40, 0.40, 0.36, 0.38, and 0.50, respectively (Fig. 4.18.). The prediction accuracy of the trait in E1, E2, E4, and E5 were 0.46, 0.43, 0.41, 0.54 respectively. As the E3 was un-replicated, thus the prediction accuracy of this environment was not calculated.

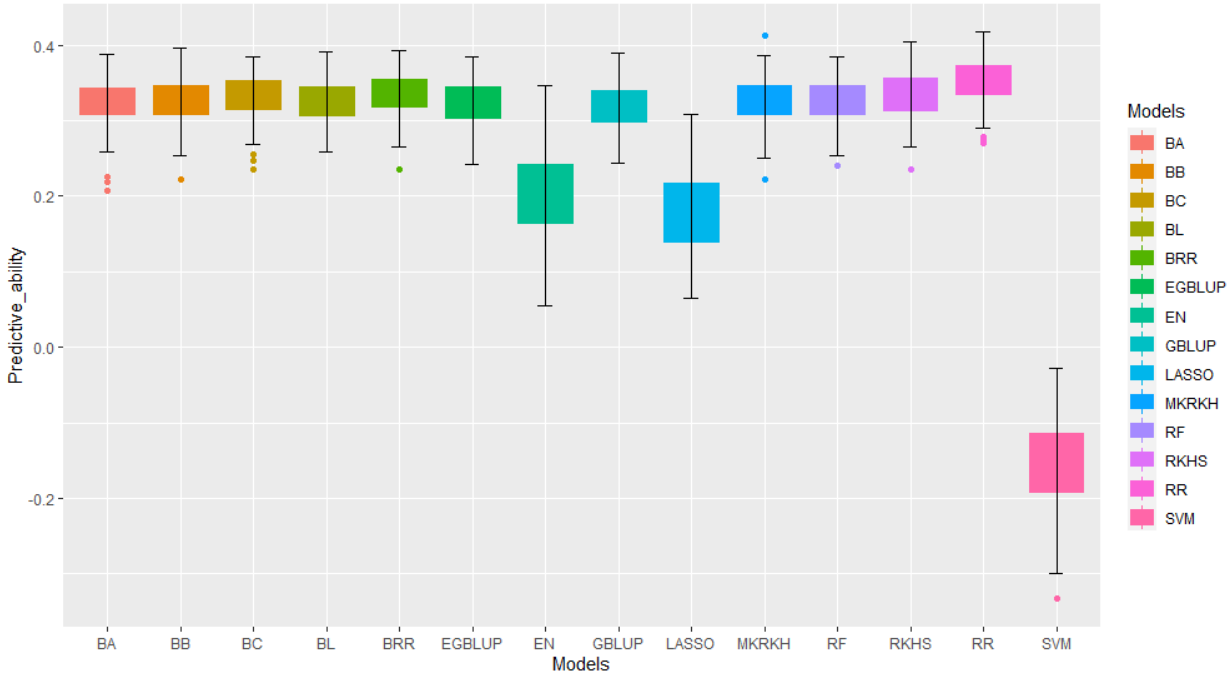


Fig. 4.13. Predictive ability of all models for canola pod shattering resistance in E1 = Osnabrock 2020

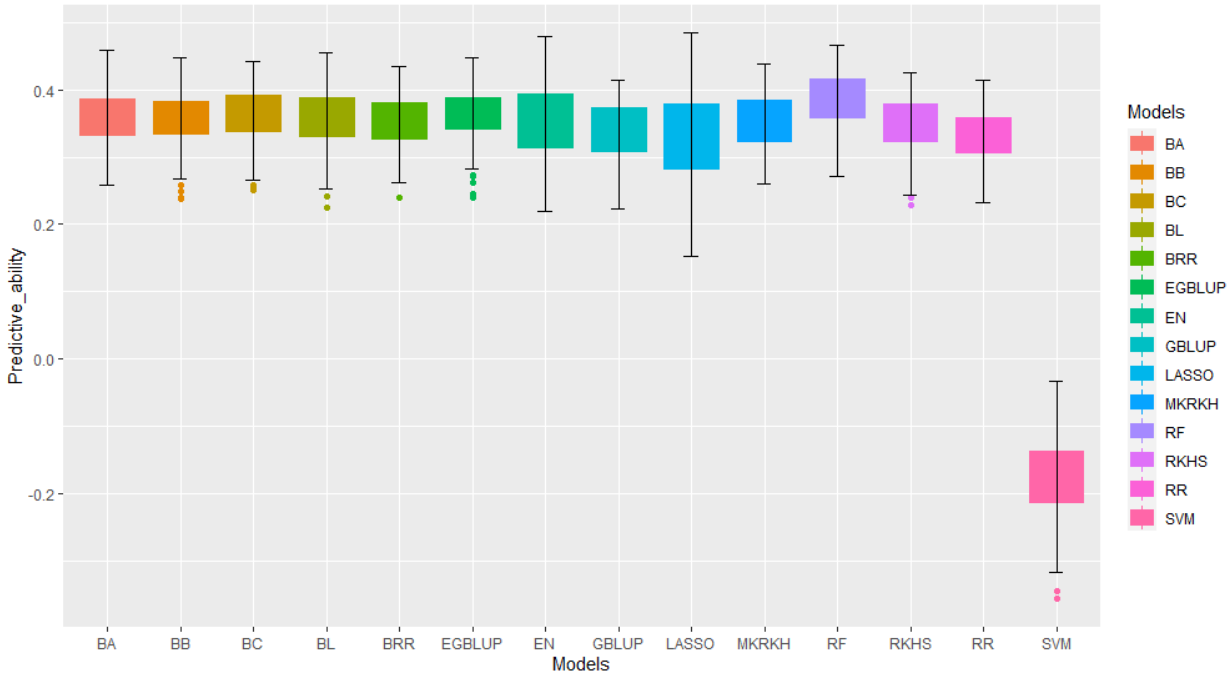


Fig. 4.14. Predictive ability of all models for canola pod shattering resistance in E2 = Fargo 2020

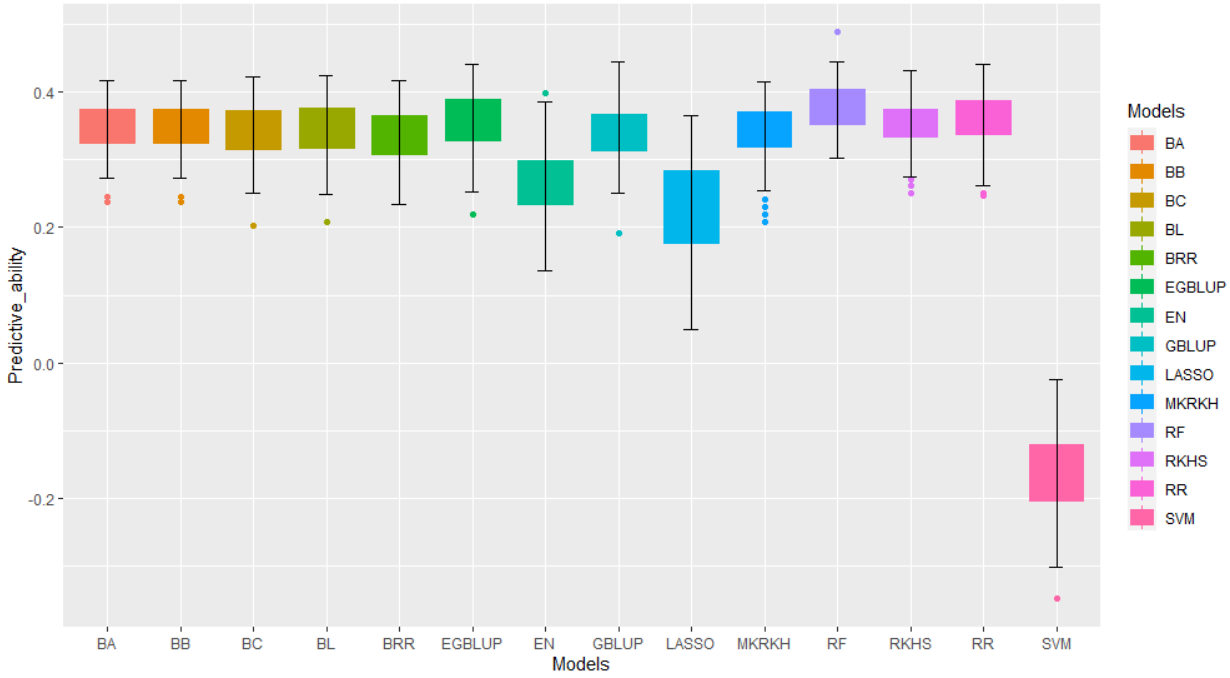


Fig. 4.15. Predictive ability of all models for canola pod shattering resistance in E3 (Carrington 2020)

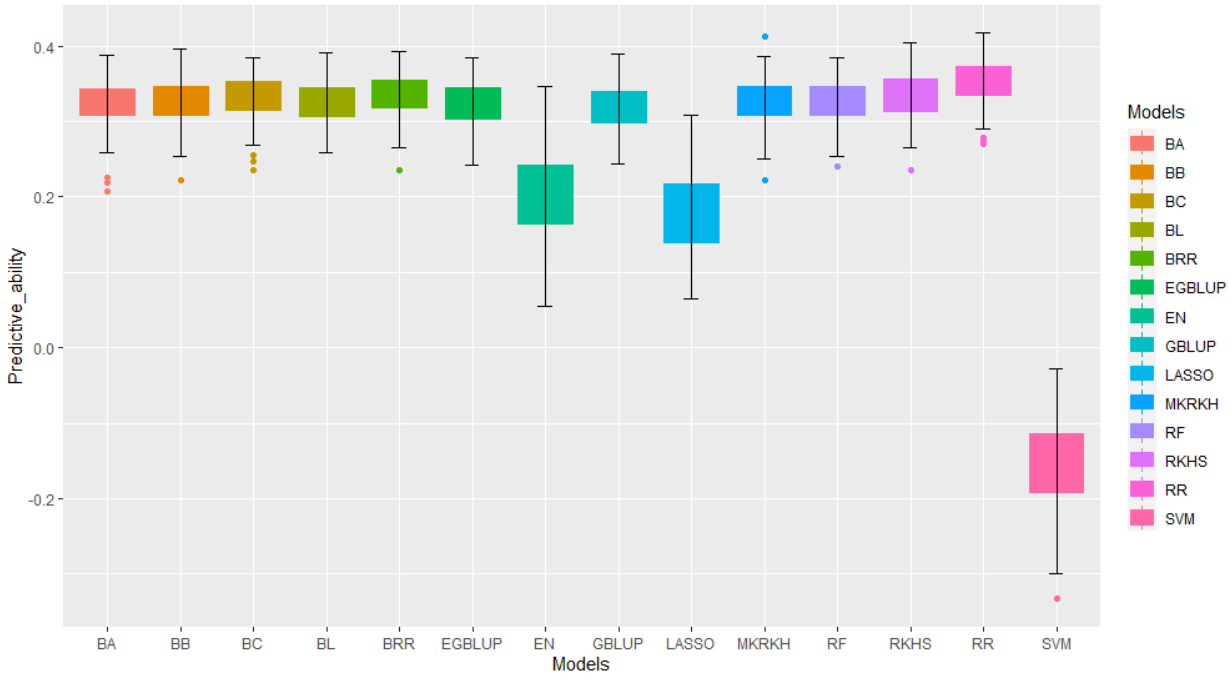


Fig. 4.16. Predictive ability of all models for canola pod shattering resistance in E4 (Osnabrock 2021)

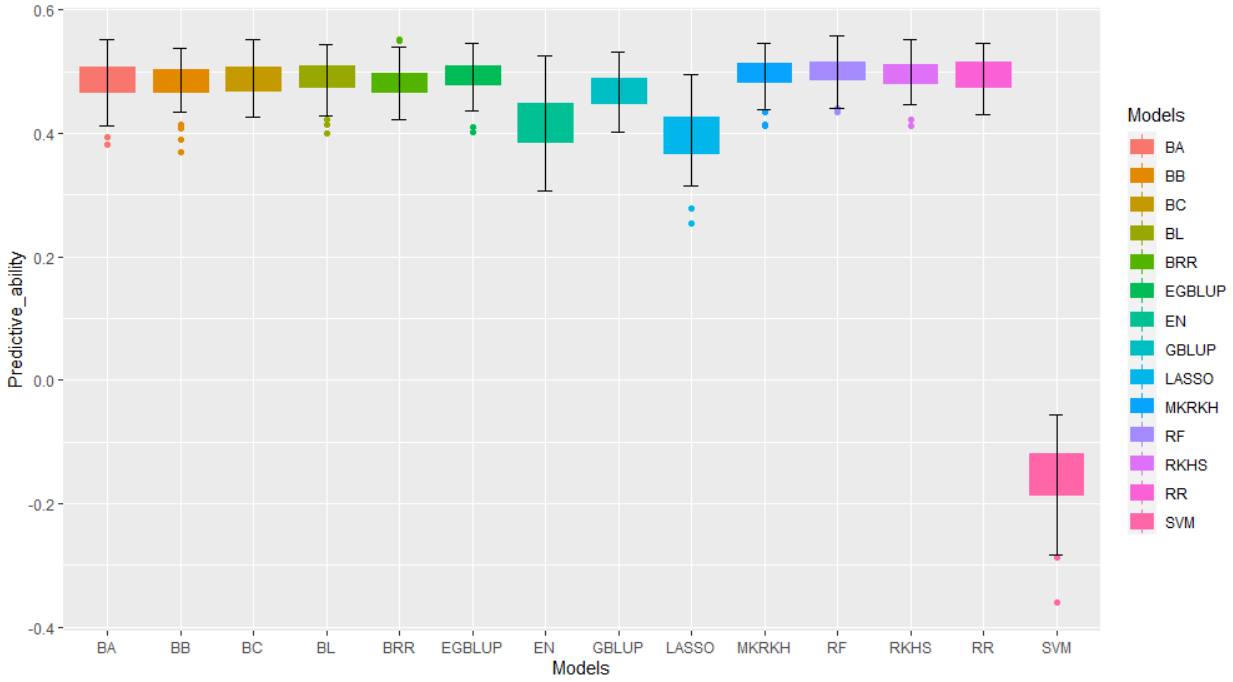


Fig. 4.17. Predictive ability of all models for canola pod shattering resistance in E5 (Greenhouse 2021)

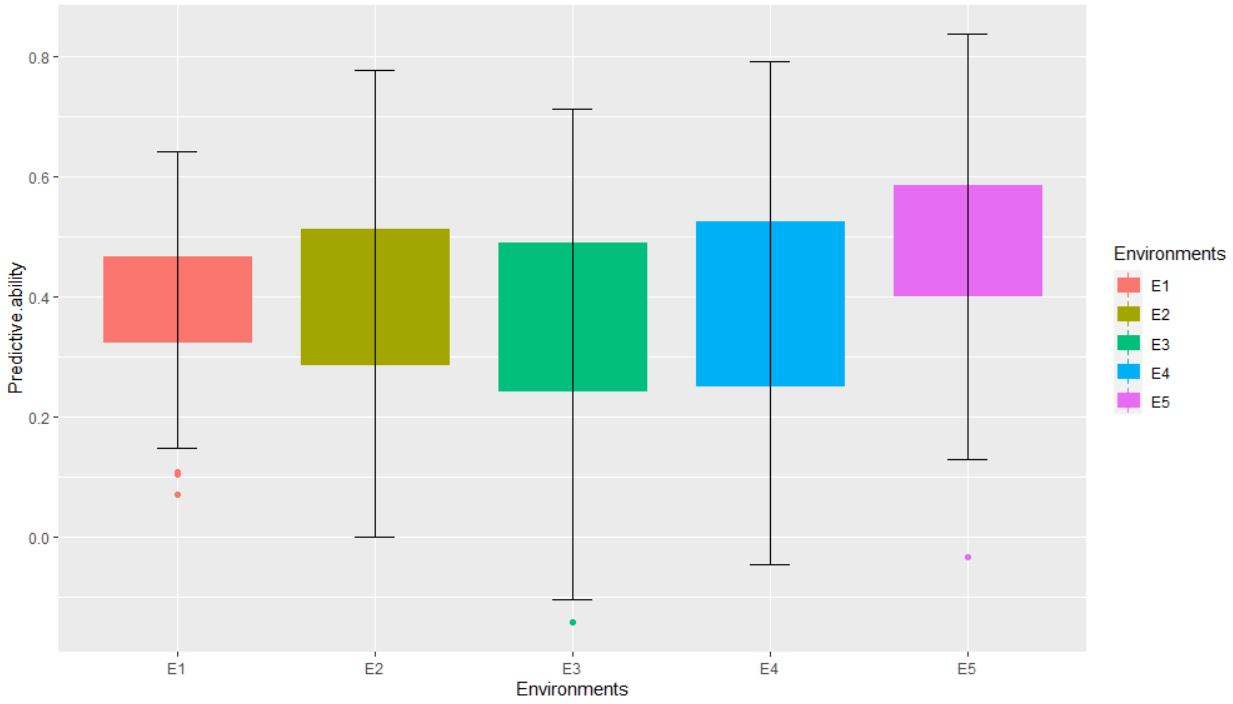


Fig. 4.18. Predictive ability of the reference model rrBLUP for canola pod shattering resistance in all environments.
Note: E1 = Osnabrock 2020, E2 = Fargo 2020, E3 = Carrington 2020, E4 = Osnabrock 2021, and E5 = Greenhouse 2021.

Table 4.4. Predictive ability and predictive accuracy of all models implemented in all environments

| GP Model | Predictive ability & predictive accuracy (in parentheses) in different Environments* | | | | |
|---|---|---|---|---|---|
| | E1 | E2 | E3 | E4 | E5 |
| BayesA | 0.32 (0.38) | 0.36 (0.39) | 0.35 | 0.37 (0.40) | 0.49 (0.53) |
| BayesB | 0.33 (0.38) | 0.36 (0.38) | 0.35 | 0.37 (0.41) | 0.48 (0.52) |
| BayesC | 0.33 (0.38) | 0.36 (0.39) | 0.34 | 0.37 (0.40) | 0.49 (0.53) |
| BL | 0.33 (0.38) | 0.36 (0.38) | 0.34 | 0.38 (0.41) | 0.49 (0.53) |
| BRR | 0.34 (0.39) | 0.35 (0.38) | 0.34 | 0.38 (0.41) | 0.48 (0.53) |
| EN | 0.21 (0.24) | 0.35 (0.38) | 0.27 | 0.36 (0.39) | 0.42 (0.45) |
| EGBLUP | 0.32 (0.38) | 0.36 (0.39) | 0.36 | 0.35 (0.38) | 0.49 (0.53) |
| GBLUP | 0.32 (0.37) | 0.34 (0.37) | 0.34 | 0.36 (0.39) | 0.47 (0.51) |
| LASSO | 0.18 (0.21) | 0.33 (0.35) | 0.23 | 0.37 (0.40) | 0.40 (0.43) |
| MKRKHS | 0.32 (0.38) | 0.35 (0.38) | 0.34 | 0.34 (0.37) | 0.50 (0.54) |
| RKHS | 0.33 (0.39) | 0.35 (0.37) | 0.35 | 0.34 (0.37) | 0.49 (0.54) |
| RF | 0.33 (0.38) | 0.38 (0.41) | 0.38 | 0.37 (0.40) | 0.50 (0.54) |
| RR | 0.35 (0.41) | 0.33 (0.36) | 0.36 | 0.36 (0.39) | 0.49 (0.54) |
| SVM | -0.16 (-0.18) | -0.18 (-0.19) | -0.16 | -0.19 (-0.21) | -0.16 (-0.17) |

* E1 = Osnabrock 2020, E2 = Fargo 2020, E3 = Carrington 2020, E4 = Osnabrock 2021, and E5 = Greenhouse 2021.

# CHAPTER 5. DISCUSSION AND CONCLUSION

Fruit dehiscence is a natural process of seed dispersal. However, in cultivated crops it is one of the major sources of yield loss. Pod dehiscence is a highly undesirable trait in canola. Dehiscent fruits make harvesting difficult and lead to significant production losses. Shattering can cause significant yield losses of up to 70% in rapeseed/canola (Raman et al., 2017; Steponavičius et al., 2019). Pod shattering is initiated in the dehiscence zone (DZ) on the pod. A dehiscence zone develops between the two valves and the replum in mature pods. DZ consist of highly differentiated cells which weaken the strength of the pods, leading seed dispersal. Genetic characteristics are major factors that affect pod shatter resistance (Kuai et al., 2016). Morgan et al., (2000) reported pod shattering resistance is not correlated with agronomic traits such as pod density, length and width of the pod, or seed number per pod. Resistance sources of pod shattering in *B. napus* is important in the canola breeding program to address the pod shattering issue in canola. Our results suggest the pod shattering trait in canola is highly heritable. Our estimated heritability of this trait ranges from 74 % to 87 % across environments, which strongly agree with the result of a previous experiment conducted by Raman et al. (2014). They reported the heritability of pod strength ranges from 73.1 5 to 89.8 % across environments.

## 5.1. Genome-wide association study

Genetic mapping is important for crop breeding and improvement which connect genotype to phenotype (Mackay, 2001). Bi-parental linkage mapping and association mappings (AM) are two common approaches for genetic mapping. AM / GWAS is a powerful tool to dissect the complex traits in plants (Kaler et al., 2020), and currently using widely over the traditional bi-parental linkage mapping in both plant and animals (Goddard and Hayes, 2009). The power of GWAS to detect marker trait association can be affected by population size,

population structure, linkage disequilibrium, genetic architecture of the trait, heritability of the trait, and statistical models used (Gupta et al., 2005; Josephs et al., 2017).

We used 8 different GWAS models to identify the markers associated with pod shatter resistance in canola. Five single locus analysis algorithms: GLM, MLM, CMLM, ECMLM, and SUPER; and three multi-locus analysis algorithms: MLMM, FarmCPU, and BLINK. Briefly, GLM reduces the false positives due to population structure by using principal components (PCs) (Price et al., 2006). MLM uses PCs and kinship matrix to control the false positives due to family relatedness and population structure (Yu et al., 2006). Compared to MLM, CMLM improves statistical power by clustering the individuals into groups and considering the genetic values of groups as random effects (Zhang et al., 2010). ECMLM uses several different algorithms to calculate kinship, and apply the best combination between kinship algorithms and grouping algorithms (Li et al., 2014). SUPER generate kinship by using the associated genetic markers instead of all the markers. It is an advanced version of FaST-Select, and it uses a bin approach to select associated markers (Wang et al., 2014). When it comes to the power of false-discovery rate and the QTL detection, MLMM performed better by using a kinship matrix and selected cofactors than a model which uses only a kinship matrix or only cofactors (Segura et al., 2012). FarmCPU is an iterative method that uses a modification of MLM, MLMM, and incorporates multiple markers simultaneously as covariates in a stepwise MLM to solve the confounding factors between testing markers and kinship. It completely removes the confounding factors by dividing the MLMM into two parts and using them iteratively; a fixed effect model and a random effect model (Liu et al., 2016). BLINK was inspired by FarmCPU. It differs from FarmCPU in two major ways. One, it eliminates the assumption that causal genes are evenly distributed across the genome. Another is the use of Bayesian information criteria

(BIC) in a fixed effect model to replace restricted maximum likelihood in the random effect model, and linkage disequilibrium information to replace the bin method (Huang et al., 2019).

We identified 21 significant SNPs associated with pod shatter resistance in *B. napus* through GWAS. These SNPs are located on chromosomes A01, A03, A05, A06, A09, A10, C02, C03, C04, C05, C08, C09 of *Brassica napus*. Among the 21 SNPs, 10 SNPs are consistent in more than one location. Hu et al. (2012) identified 70 significant SNPs associated with pod shattering in a panel of 276 F2 *B. napus* individuals derived from a bi-parental cross. They found a cluster of SNPs on Chromosome A09 and suggested a major QTL for pod shatter resistance reside in the region. Raman et al. (2014) identified 12 QTL on chromosomes A03, A07, A09, C03, C04, C06, and C08 in a panel of 126 double haploid populations. To improve the efficiency of QTL detection as well as to identify reliable and stable QTL for pod shatter resistance, Liu et al. (2016) used both QTL and GWAS approaches. They identified 6 QTLs on chromosome A01, A06, A07, A09, C02 and C05 from 143 *B. napus* accession, double haploid, and inter-mated $F_2$ population. These previous findings and our experimental results reveal one common linkage group associated with pod shattering in *B. napus* on A09. Although the SNPs detected on chromosome A09 are not the same SNPs among those studies, the results of these four studies suggest that at least one locus on chromosome A09 control pod shattering in *B. napus*. Kaur et. al., (2020) detected 23 SNPs associated with pod shatter resistance in *Brassica juncea*. The SNPs were found in both the A and B genomes of *B. Juncea*. They also reported SNPs on chromosome A09 for pod shatter resistance in *B. Juncea*, which further suggest that chromosome A09 is important for pod shattering in *Brassica* species. In ours and in these other studies, associated SNPs on A09 were mapped to SCM002767.2_36584139 (SNP position 36584139), SCM002767.2_36571589 (SNP position 36571589), Bn-A09-p30171993 (SNP position

34170386), Qrps.wwai-A09b (QTL interval 3089525/3155356), Qrps.wwai-A09b (QTL interval 3155356/3104590), a cluster of 14 SNPs on A09 with chromosomal position range from 29950101 – 30345915, and seven SNPs within the range of chromosomal position from 30954374 – 31232087 on A09. This suggests a potential locus near 29950101 - 36584139 position on A09 likely plays a significant role in pod shattering.

### 5.2. Candidate genes

*B. napus* has a limited genetic diversity for shatter resistance genes (Raman et al., 2014). Lack of pod shatter resistant germplasm in *B. napus* highly restricts the canola production (Li et al., 2021). Several genes involving in the development and differentiation of valve, replum and dehiscence zone have been identified previously which includes regulatory transcription factors (TFs), downstream metabolic genes, and cellulases and hemicellulases (Liljegren et al., 2000, 2004; Dong and Wang, 2015; Roeder et al., 2003; He et al., 2018; Li et al., 2021).

In our study, three candidate genes, *IND*, agamous-like MADS-box protein AGL65 (*AGL65*), and *MANNANASE7* (*MAN7*), all within the 50 kb upstream and downstream of the significant SNPs were identified in this experiment for pod shatter resistance in canola. One basic helix-loop-helix (bHLH) transcription factor *IND*, which encodes bHLH protein, was found on chromosome A03 of *B. napus*. *IND* is necessary for pod dehiscence (Wu et al., 2006; Van Gelderen et al., 2016) and it acts downstream of *SHP1/2* to control pod shattering in Arabidopsis (Liljegren et al., 2000, 2004). *IND* and another bHLH transcription factor *ALC*, they both play important role in pod dehiscence in Arabidopsis, act down-stream of and in parallel with *SHP1/2* (Dong and Wang, 2015). Zhai et al. (2019) reported *IND* is highly conserved in *Brassica* species. They also reported *IND* is essential for pod shatter resistance while *ALC* gene has limited ability to alter pod shatter resistance in *B. napus*. *SHP1* and *SHP2* control pod

shattering in Arabidopsis (Liljegren et al., 2000). The *SHP1and SHP2* genes are two MADS-box transcription factors encoding genes. We found the *AGL65* gene on chromosome C03 which is a member of MCMl AGAMOUS DEFICIENS SRF box (MADS-box) gene family. MADS-box transcription factors are involved in several aspects of plant development (Rounsley et al., 1995; Parenicová et al., 2003). *AGL65* regulates pollen activity and is expressed in pollen (Adamczyk and Fernandez 2009). One protein coding gene *mannan endo-1,4-beta-mannosidase 3-like* was found on chromosome C04, which is similar to the *mannan endo-1,4-beta-mannosidase 7* protein encode *MAN7* gene in *B. napus*. The *MAN7* genes involved in the fruit dehiscence, fruit valve development, plant-type cell wall loosening (He et al., 2018), and seed germination (Iglesias-Fernández et al., 2011) in *Arabidopsis thaliana*. Mannans are hemicellulases (Iglesias-Fernández et al., 2011), and both cellulases and hemicellulases are important for fruit development and maturation (He et al., 2018). *MAN7* and a cellulase gene CELLULASE6 (CEL6) are expressed in vegetative and reproductive organs and their expression partially depends on transcription factors *IND* and *ALC*. He et al., 2018 demonstrated, in *Arabidopsis thaliana, MAN7* and *CEL6* genes function in the siliques development and dehiscence. The function of the *MAN7* gene in *B. napus* in silique dehiscence has been verified by Li et al., (2021). They cloned the homolog of Arabidopsis's *MAN7* from *B. napus* and showed its function in silique dehiscence in rapeseed. Their study confirms that the *MAN7* gene in *B. napus* encodes a hemicellulose and by altering its expression dehiscence resistance can be manipulated. Li et al., (2021) also revealed that down-regulation of *MAN7* gene significantly increases the pod dehiscence-resistance in *B. napus*.

### 5.3. Genomic prediction

Genomic selection (GS) is an effective genomic strategy for crop improvement specially to improve complex traits with a hope to capture minor-to medium effect loci (Meuwissen et al., 2001). It is a cost-effective approach which reduces the breeding cycles and allows the breeder to select the best parents for crossing or cultivar development for quantitative traits. It accelerates breeding progress by increasing genetic gains. Until now, with our best knowledge, genomic prediction (GP) for pod shattering in canola has not been reported yet. However, genomic prediction on different traits such as for stress tolerance, disease resistances, and others in canola has been reported. Differences in the population size and genetic diversity, linkage disequilibrium extent, heritability of the traits might lead to the differences in the predictive ability (Daetwyler et al. 2010; Crossa et al. 2017).

We used 14 genomic prediction models in our study including GBLUP (VanRaden, 2008; Habier et al., 2013), EGBLUP (Jiang and Reif, 2015), rrBLUP (Meuwissen et al., 2001; Endelman, 2011), LASSO (Tibshirani, 1996; Usai et al., 2009), EN (Zou and Hastie, 2005), BRR (Gianola et al., 2003; Desta and Ortiz 2014), BL (Park et al., 2008), BA (Habier et al., 2011; Meuwissen et al., 2001) , BB (Habier et al., 2011; Meuwissen et al., 2001), BC (Habier et al., 2011; Meuwissen et al., 2001), RKHS (Gianola and Van Kaam, 2008; De Los Campos et al., 2010), MRKHS (Gianola and Van Kaam, 2008; De Los Campos et al., 2010), and SVM (González-Recio et al., 2014), RF (Breiman et al., 2001). Briefly, markers effects are normally distributed and have identical variance in rrBLUP (Meuwissen et al., 2001). A modification of the conventional BLUP is GBLUP, which uses a marker-based relationship matrix (VanRaden, 2008; Habier et al., 2013). While EGBLUP is BLUP which uses a “squared” relationship matrix to model epistatic 2x2 interactions (Jiang and Reif, 2015). BA and BB model are similar.

However, markers effects have different variances in BA, whereas BB have some of the marker effects with zero variance. In BC, markers have normally distributed effects (Meuwissen et al. 2017). Marker effects are estimated from a double exponential distribution in the BL model, and it applies both shrinkage and variable selection (De Los Campos et al., 2009). A Gaussian distribution of the marker effects is produced in the BRR model while creating equal shrinkage of all the marker effects to zero (Desta and Ortiz 2014). Genetic distance and a kernel function-based model RKHS and MRKHS, which are used to regulate the distribution of marker effects Gianola and Van Kaam, 2008; De Los Campos et al., 2010). SVM is a machine learning method for classification, regression, and others learning related task (González-Recio et al., 2014). RF is capable of capturing interactions between markers, and it is based on regression models (Breiman et al., 2001). LASSO is a penalized regression method. In LASSO, more marker effects will shrink to zero than ridge regression (Tibshirani, 1996; Usai et al., 2009).

These models were implemented in the BWGS pipeline in R, described by (Charmet et al., 2020). Briefly, BWGS (BreedWheat Genomic Selection pipeline) is an integrated pipeline which was developed using available R functions in different R libraries (Charmet et al., 2020). The BWGS pipeline consists of two main functions ‘bwgs.cv’ and ‘bwgs.predict’. The function ‘bwgs.cv’ uses genotype and phenotype data to cross validate the model on a training set. The ‘bwgs.predict’ function calibrate the model on a training set, and predict the GEBV (Genomic Estimation of Breeding Values) of a target population by applying the model on the genotyping data of the target population (Charmet et al., 2020).

Koscielny et al. (2020) reported GP ranges from 0.14 to 0.66 among the nine different traits evaluated for heat-stress tolerance in canola. Where the lowest GP ability was 0.14 for yield and highest was 0.66 for 1000-seed weight using GBLUP and rrBLUP. Fikere et al. (2020)

evaluated genomic prediction on different diseases, agronomic, and seed quality traits and found prediction accuracy ranges from 0.29 to 0.69, where 0.29 was for emergence count and 0.69 for seed yield. Roy et al. (2021) reported genomic prediction ability ranges from 0.41 to 0.64 for the four traits investigated for Sclerotinia stem rot resistance in canola, evaluated in a panel of 187 canola germplasm phenotyped in the field. Another study conducted by Roy et al. (2022), reported 0.45 to 0.68 genomic prediction ability for sclerotinia stem rot resistance in canola, evaluated in a panel of 337 canola germplasm phenotyped in the greenhouse.

In our study, among the 14 GP models, SVM produces the poorest and negative predictive ability. Charmet et al. (2020) also found SVM being the worst GP model among others. One of the reasons behind this could be the higher number of markers used for this analysis. Charmet et al. (2020) found the SVM model is comparable when relatively low amounts (around 5000) of markers are being used, however SVM seems unable to handle high amounts of markers. As we used more than 20,000 high quality markers in our study in each environment for genomic prediction, SVM seems unable to deal with this markers volume. Hence, the result from SVM will be discarded. The predictive ability of other 13 models range from 0.18 – 0.50 across the environments. The RF and MKRKH models gave the highest predictive ability of 0.50 in the environment E5. Where the lowest predictive ability was obtained from the LASSO model which gave 0.18 in the environment E1. This result is highly similar to the study conducted by Charmet et al. (2020). They also found RF gives the higher predictive ability while LASSO being the lowest.

In addition to implementing the 14 GP models in the BWGS pipeline, we performed a separate genomic prediction on pod shatter resistance in canola using rrBLUP as a reference GP model, regardless of the RR was included as one of the 14 models in the BWGS. The predictive

ability from the reference model rrBLUP ranged from 0.36 – 0.50 across environments, and the predictive accuracy ranges from 0.41 – 0.54 across environments. The predictive ability from the reference model is highly correlated with the predictive ability obtained from the 13 models implemented in the BWGS pipeline. None of the GP models implemented in the BWGS pipeline were able to outperform the performance of the reference model rrBLUP. This result suggests that keeping rrBLUP as a reference model for genomic prediction is reasonable. The results of this study suggest that the genomic selection holds potential to improve pod shatter resistance in canola. A genomic prediction ability of 0.50 with a prediction accuracy of 0.54 for pod shatter resistance in canola, could be fitted into a moderate to high predictive ability range considering the complex nature of the trait.

### 5.4. Conclusion

We have evaluated a total of 150 spring type *B. napus* genotypes in five different environments including field and greenhouse conditions. This study identified 21 significant SNPs associated with pod shatter resistance in *B. napus*. The study suggested that at least one locus on chromosome A09 involved in pod shattering in *B. napus*. The study also suggests that *IND* is a major gene possibly controlling pod shattering in canola. In addition, genes from the *AGAMOUS* gene family and gene from the *MANNANASE* gene family play an important role in pod shattering in *B. napus*. We implemented 14 genomic selection models to test their ability of genomic prediction for pod shatter resistance in canola. The genomic prediction ability for this trait went up to 0.50 and prediction accuracy went up to 0.54. The experiment suggests that the genomic selection might be beneficial to apply in the canola breeding program for effective selection of genotype for pod shatter resistance.

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## APPENDIX A. ANALYSIS OF VARIANCE (ANOVA) OF POD SHATTER RESISTANCE IN DIFFERENT ENVIRONMENT

Osnabrock 2020

| Source of variation | df | Mean square | F value | *P* value | $H^2$ |
|---|---|---|---|---|---|
| Replicates | 2 | 2.35 | $0.24^{NS}$ | 0.79 | 0.74 |
| Genotypes | 139 | 37.20 | $3.81^{***}$ | <.0001 | |
| Genotypes x Replicates /Exp. Error | 278 | 9.77 | | | |

Fargo 2020

| Source of variation | df | Mean square | F value | *P* value | $H^2$ |
|---|---|---|---|---|---|
| Replicates | 2 | 21.40 | $3.79^{*}$ | 0.024 | 0.87 |
| Genotypes | 128 | 42.35 | $7.50^{***}$ | <.0001 | |
| Genotypes x Replicates /Exp. Error | 256 | 5.64 | | | |

Osnabrock 2021

| Source of variation | df | Mean square | F value | *P* value | $H^2$ |
|---|---|---|---|---|---|
| Replicates | 2 | 12.64 | $1.86^{NS}$ | 0.16 | 0.86 |
| Genotypes | 94 | 56.71 | $8.34^{***}$ | <.0001 | |
| Genotypes x Replicates /Exp. Error | 188 | 6.80 | | | |

Greenhouse 2021

| Source of variation | df | Mean square | F value | *P* value | $H^2$ |
|---|---|---|---|---|---|
| Replicates | 2 | 210.28 | $26.2^{***}$ | <.0001 | 0.86 |
| Genotypes | 133 | 58.79 | $7.33^{***}$ | <.0001 | |
| Genotypes x Replicates /Exp. Error | 266 | 8.02 | | | |

Note:

$^{NS}$ Differences were non-significant at $P < 0.05$ levels of significance

* Differences were significant at $P < 0.05$ levels of significance

*** Differences were significant at $P < 0.0001$ levels of significance

## APPENDIX B. LIST OF TOTAL *BRASSICA NAPUS* GENOTYPES USED IN THIS STUDY

| Name of the accession | PI number | Country |
|---|---|---|
| Aviso | #N/A | Canada |
| Bingo | PI 546468 | USA |
| BO-63 | Ames 15651 | Canada |
| Brio | PI 458919 | France |
| Bronowski | PI 469737, Ames 22548, PI 649132 | Poland |
| Buk Wuk 3 | PI 469738 | South Korea |
| Celebra | PI 538766 | Sweden |
| Ceskia Tabor | Ames 2793 | Czech Republic |
| Colt | PI 633119 | USA |
| Colza | PI 469756 | South Korea |
| Comet | Ames 15939 | Sweden |
| Conquest | Conquest (RR) | Canada |
| Cougar | Cougar (IMI) | Canada |
| Cresor | #N/A | France |
| Cresus | #N/A | France |
| Crop | PI 458922 | France |
| Czyzowski | PI 535847, PI 311728 | Poland |
| Delta | PI 543937 | Sweden |
| Drakkar | #N/A | France |
| Evvin | PI 633131 | Russian Federation |
| Flint | PI 605719 | USA |
| Fonto | PI 469789 | South Korea |
| France 1 | PI 469791 | France |
| Galant | #N/A | USA |
| Galaxy | Ames 15938 | Sweden |
| Gido | PI 458946 | Germany |
| Gisora | PI 458948 | Germany |
| Global | PI 601200 | Sweden |
| Golden | PI 649126 | Canada |
| Gora | PI 458949 | Germany |
| Gulle | PI 458936 | Sweden |
| Gullivar | PI 458937 | Sweden |
| Hi-Q | #N/A | Canada |
| INRA-R-2000 | #N/A | France |

| Name of the accession | PI number | Country |
|---|---|---|
| IR-2 | PI 531280 | Hungary |
| Janetzkis | PI 469826 | South Korea |
| Jasna | #N/A | Serbia |
| Kanada | #N/A | Poland |
| Klinki | PI 469840 | South Korea |
| Kosa | PI 458951 | Germany |
| Koubun | PI 469841 | South Korea |
| Kovalevskjj | PI 633132 | Ukraine |
| Kraphhauser | PI 469842 | South Korea |
| Kritmar rape | PI 469843 | South Korea |
| Laura | PI 458952 | Germany |
| Legend | PI 633118 | Sweden |
| Lifura | PI 469888 | South Korea |
| Mar'janovskij | PI 633125 | Ukraine |
| Mazowiecki | #N/A | Poland |
| Midas | PI 431571 | Canada |
| Miekuro Dane | PI 469901 | South Korea |
| Mura yamasho | PI 469941 | South Korea |
| NDC-A14026 | DH010 | USA |
| NDC-A14032 | DH067 | USA |
| NDC-A14033 | A07-25NR (M) | USA |
| NDC-A14035 | (VisxKan) | USA |
| NDC-A14036 | (KanxVis) | USA |
| NDC-A14045 | Regentx Lagoda | USA |
| NDC-A14046 | Regent x Lagoda | USA |
| NDC-A14050 | Regent x Lagoda | USA |
| NDC-A14055 | Kan x Fashion | USA |
| NDC-A14056 | Fashion x Kan | USA |
| NDC-E12009 | 16-1013 (DL Seeds-8) | USA |
| NDC-E12023 | VisionXKanda | USA |
| NDC-E12025 | (VisxKan)X11-1977 | USA |
| NDC-E12027 | (VisxKan)X11-1977 | USA |
| NDC-E12044 | VisionXKanda | USA |
| NDC-E12079 | Cometx BC-31 | USA |
| NDC-E12081 | A04-74NAx Comet | USA |
| NDC-E12086 | KandaXFavorite | USA |
| NDC-E12119 | FavoriteXKanda | USA |
| NDC-E12120 | KandaXFavorite | USA |

| Name of the accession | PI number | Country |
|---|---|---|
| NDC-E12121 | KandaXFavorite | USA |
| NDC-E12131 | KandaXFavorite | USA |
| NDC-E12133 | FavoriteXKanda | USA |
| NDC-E13193 | F2 FavoritexKanda | USA |
| NDC-E13279 | | USA |
| NDC-E13285 | | USA |
| NDC-E15031 | 1 RA-1577 WS BC | USA |
| NDC-E15146 | 1 RA-1755 RUT | USA |
| NDC-E15174 | 1 RA-1761 RUT | USA |
| NDC-E15200 | 1 RA-1767 RUT | USA |
| NDC-E15234 | 1 RA-1774 RUT | USA |
| NDC-E15294 | (VisxKan)X11-1977 | USA |
| NDC-E16015 | (Vision x Kanada) x (Vision x Kanada) | USA |
| NDC-E16053 | (Vision x Kanada) x (Vision x Kanada) | USA |
| NDC-E16152 | (Vision x Kanada) x [N12-989 (Kanada x Fashion)] | USA |
| NDC-E16169 | (Vision x Kanada) x (Vision x Kanada) | USA |
| NDC-E16198 | (Vision x Kanada) x [N12-989 (Kanada x Fashion)] | USA |
| NDC-E17132 | (NDSU9-1013 x ((KanxVis)] x (Winxsp-field) | USA |
| NDSU01104 | A01-104NA | USA |
| NDSU0417 | A05-17NI | USA |
| NDSU0472 | A04-72NA | USA |
| NDSU0473 | A04-73NA | USA |
| NDSU0474 | A04-74NA | USA |
| NDSU0475 | A04-75NA | USA |
| NDSU0521 | A05-21NA | USA |
| NDSU0522 | A05-22NA | USA |
| NDSU0619 | A06-19NA | USA |
| NDSU0620 | A06-20NA | USA |
| NDSU0726 | A07-26NR | USA |
| NDSU0728 | A07-28NA | USA |
| NDSU0729 | A07-29NI | USA |
| NDSU10999 | A-10-999 | USA |
| NDSU12989 | A-12-989 | USA |
| NDSU151000 | A-15-1000 | USA |
| NDSU15989 | A-15-989 | USA |
| NDSU161013 | A-16-1013 | USA |

| Name of the accession | PI number | Country |
|---|---|---|
| NDSU31001 | A-3-1001 | USA |
| NDSU31011 | A-3-1011 | USA |
| NDSU41000 | A-4-1000 | USA |
| NDSU7997 | A-7-997 | USA |
| NDSU81000 | A-8-1000 | USA |
| NDSU91013 | A-9-1013 | USA |
| NU 41737 | PI 649135 | Turkey |
| NU 51084 | PI 633124 | Sweden |
| Oro | PI 458930 | Canada |
| Orpal | PI 458968 | France |
| Peace | #N/A | Canada |
| Polo canola | Ames 26635 | USA |
| Premier | PI 639274 | USA |
| Printol | PI 552810 | USA |
| Prota | PI 458955 | Germany |
| Q2 | #N/A | Canada |
| R2000 | | Franch |
| Ratnik | #N/A | Serbia |
| Regent | PI 431572 | Canada |
| Regina II | Ames 1669 | Canada |
| Reston | PI 649152 | USA |
| Rico | PI 458956 | Germany |
| Romeo | PI 458971 | France |
| Russia 5 | PI 470021 | Russian Federation |
| S.V. Gulle | PI 470032 | South Korea |
| Seoul | PI 537090 | South Korea |
| Silex | #N/A | Canada |
| Sunrise | PI 597352 | USA |
| Sval of Gullen | PI 470033 | South Korea |
| Taiwan | PI 470039, PI 470038 | Taiwan |
| Tanto | #N/A | France |
| Target | PI 458926, PI 470045 | Sweden |
| Tobin | Ames 26654 | USA |
| Tonus | PI 470050 | South Korea |
| Topas | PI 601201 | Sweden |
| Tower | PI 431574, Ames 2792, PI 431574 | Canada |
| Turret | PI 365644 | Canada |

| Name of the accession | PI number | Country |
|---|---|---|
| Vostochno-sibirskii | PI 633126 | Russian Federation |
| Wasefuji | PI 470054 | South Korea |
| Westar | Ames 26653 | Canada |
| Willa | PI 470058 | South Korea |
| Winfield | | USA |