EXPLORING QUANTITATIVE TRAIT LOCI MAPPING FOR BUD FRUITFULNESS AND BUD BREAK TRAITS IN GRAPEVINE F2 POPULATION

BY

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THESIS ACCEPTANCE PAGE

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This thesis is approved as a creditable and independent investigation by a candidate for the master's degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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I would like to dedicate my thesis to my parents, Rahime Gunes and Zekeriya Gunes.

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ABBREVIATIONS

%	Percentage
°C	Degree celsius
AFLP	Amplified fragment length polymorphism
AUBPC	Area under bud break progression curve
BF	Bud Fruitfulness
Chr	Chromosome
CI	Confidence interval
CIM	Composite interval mapping
DNA	Deoxyribonucleic acid
F1	First filial generation
F2	Second filial generation
GBS	Genotype-by-sequence
LOD	Likelihood of odds
MAS	Marker-assisted selection
N	North
NGS	Next-generation sequence
p-value	Probability value
QTL	Quantitative trait loci
R	R statistical software
RAPD	Random amplification of polymorphic DNA
RFLP	Restriction fragment length polymorphism
RhAmpSeq	RNase H2 enzyme-dependent amplicon sequencing
RRL	Reduced representation library
SNP	Single nucleotide polymorphism
SSR	Simple sequence repeats
SIM	Simple interval mapping

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ABSTRACT

EXPLORING QUANTITATIVE TRAIT LOCI MAPPING FOR BUD FRUITFULNESS AND BUD BREAK TRAITS IN GRAPEVINE F2 POPULATION

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Grapevine (*Vitis vinifera* L. subsp. *vinifera*) is one of the most important and valuable fruit crops around the world and grape industries in the USA have been growing increasingly regarding the demand for highly productive and quality grapes that can be grown in the cold region around the USA. The development of new cultivars with these features is performed through grape breeding with the help of quantitative trait loci (QTL) mapping, marker-assisted selection (MAS), and and other technologies. In this study, we evaluated grapevine bud fruitfulness by position and bud break in controlled conditions. These traits are multi-genetic and understanding the genetics mechanism behind the complex traits will help to discover the underlying genes. The objectives of this thesis were to identify phenotypic variation in these traits and reveal QTL. A subset of 179 genotypes from a F_2 mapping population developed from the self of a single F_1 (16_9_2) derived from a cross between *V. riparia* and 'Seyval' were used. The F2s were grown in the greenhouse for bud fruitfulness and bud break study. Bud fruitfulness was determined, and an integrated genotype by sequencing (GBS) and rhAmpSeq linkage map (2519 markers) and standard interval mapping (SIM) were used to identify QTL. Two main QTL related to bud fruitfulness were identified, explaining 25.5 % of phenotypic variation on chromosome 4 and 5.

Bud break phenology was studied to identify the rate of bud break QTL in the F_2 genotypes. Bud break occurs after the chilling requirement is fulfilled and optimal growth conditions promote bud break. Six one-node cuttings of each genotype were forced under greenhouse conditions for thirty days and the growth stage of cuttings measured based on Modified Eichhorn-Lorenz (Modified E-L) phenology scale was monitored daily. Data were processed using the area under the curve concept (area under bud break progression curve, AUBPC) to capture the rate of bud break values for each genotype. SIM was conducted with the average AUBPC, bud break score at the third week, and bud break rate using R/qtl, 1000 permutations and the integrated GBS and rhAmpSeq linkage map. A major QTL was identified on chromosome 10 and explained 11.04% of the genotypic variation.

Bud break and bud fruitfulness studies identified several QTL and these QTL can provide candidate genes that may be used further to dissect the mechanisms underlying bud break and fruitfulness for sustainable production of grapevine.

Keywords: Grapevine, bud fruitfulness, bud break, QTL

CHAPTER 1: INTRODUCTION

1.1 GRAPE USES AND IMPORTANCE

Grapes are considered one of the major fruit crops in the world based on hectares planted and economical value (AL-OBEED *et al.* 2010; TORREGROSA *et al.* 2015). In 2018, there were 7.9 million hectares of vineyards around the world and five countries represent 50% of the world vineyard, Spain with 13%, China with 12%, France with 11%, Italy with 9%, and Turkey with 6%. Wine is by far the major product of grapes, the rest is consumed as table grape and dried into raisins (CARMONA *et al.* 2008; MYLES *et al.* 2011; TORREGROSA *et al.* 2015). According to statistics from the International Organization of Vine and Wine (OIV) (http://www.oiv.int/), grape world production was around 77.8 million tons in 2018, wine, table grape, and dried production was estimated at 57%, 36%, 7% of total production respectively. China is the largest producer of grapes followed by Italy, the USA, Spain, and France. Interestingly, the USA was the 6th country in terms of the vineyard area, but it was the third-largest producer with 6.9 million tons in 2018. Moreover, Grapes are the highest value fruit crop in the US and the grape industry contributed approximately 6.46$ million to the US economy in 2017. Although many states in the USA contribute grape production California is the largest grape and wine-producing state accounting for about 85% of the country's total output followed by Washington and New York (https://www.usda.gov/).

1.2 DOMESTICATION PROCESS OF GRAPEVINE

Domestication is the most significant genetic process of selection that transformed wild vines into domesticated crops modifying morphological and genetic characters (GRASSI AND ARROYO-GARCIA 2020). The cultivated grapevine (*Vitis vinifera* L.), one of

60 species of *Vitis* genus, is the major species in the industry of wine and table grape (ADAM-BLONDON *et al.* 2004; REISCH *et al.* 2012). Archaeological findings suggest that the domestication of grapevine started 6,000 to 8,000 years ago in the Mediterranean region of South and East Europe and it is considered to have been domesticated from its wild ancestor *Vitis vinifera* L. subsp. *sylvestris* (Gmelin, Hegi) (ARADHYA *et al.* 2003; REYNOLDS 2015; GRASSI AND ARROYO-GARCIA 2020). As the grapevine industry and wine production increased, *V.vinifera* became the most used species. However, due to lack of its resistance to pest and disease, breeders searched wild grapevine species, namely North America and East Asia with 28 and 30 species, respectively (MYLES *et al.* 2011; WAN *et al.* 2013). These species have been widely used for breeding purposes. In particular, *V. riparia*, *V. rupestris*, and *V. berlandieri* from North American species have been used by breeders to develop rootstock cultivars and in scion breeding programs to develop cultivars with high adaptability to biotic and abiotic stress with high quality and desirable time of ripening (REISCH *et al.* 2012). Among these species, *V. riparia* is one of the most commonly used species in rootstocks and scion breeding for its freezing tolerance, phylloxera resistance, and disease resistance such as powdery mildew and downy mildew and it has the greatest distribution of the North American species (HEMSTAD AND LUBY 1998; LOWE AND WALKER 2006). Since the domestication of grapevine, increased climatic problems and pest and disease problems lead to breeders to use North American species with the use of new technologies such as marker-assisted selection (MAS).

1.3 TRAITS OF INTERESTED IN NORTHERN GRAPE BREEDING

During the nineteenth century, breeding programs in grapevine began predominantly in North America (ARNOLD AND SCHNITZLER 2020). *V. vinifera* vines failed

to grow due to severe frost damage and the destruction of the grapes by pests and fungal disease thus, native American species have been used to develop interspecific cultivars (EIBACH AND TÖPFER 2015). Today, these cultivars provide a very valuable resource for grapevine breeding programs around the world (EIBACH AND TÖPFER 2015). Since the beginning of systematic grapevine breeding the main traits targeted to improve grapevines, as in the other fruit crops, are yield, quality of the grape, and resistance to disease and pests (COSTANTINI *et al.* 2008; EIBACH AND TÖPFER 2015). These components are complex and depend on multiple important grapevine traits. Yield is determined by several factors in grapevines, such as fruit size and bud fruitfulness. Fruitfulness refers to the number of clusters per shoot which is a major component of final yield (DOLIGEZ *et al.* 2010; GRZESKOWIAK *et al.* 2013). Thus, bud fruitfulness becomes a major target of grapevine breeding (EIBACH AND TÖPFER 2015). Moreover, quality in grapevine can be affected by several factors such as timing of bud break, flowering time, and berry chemical composition (EIBACH AND TÖPFER 2015). Timing of bud break impacts vegetative growth of vines as early bud break can result in frost damage in young shoots. This can also affect the quality of fruit and yield. Therefore, one of the most important goals of breeding programs in the Northern USA is to increase fruit quality, cold hardiness, and resistance to disease and pest of wine and table grape cultivars.

1.4 GRAPEVINE PHENOLOGY

To accomplish the best possible production from the grapevine, good information on grapevine phenology during the growing season is needed. The modified E-L bud scoring system produced initially by Eichhorn and Lorenz, then developed by Coombe

(1995) can be utilized to show the different phenological stages of grapevine (COOMBE 1995; GILLIAN 1996; BENNETT 2002).

Budburst: During spring, bud burst initiates when temperature reaches 10 °C and the progressive phases of bud burst are defined by E-L stages two-five. Grapevines use the carbohydrates that are stored in roots, trunks, and canes until leaves reach 50% of their final size to do photosynthesis. Finally, the overall number of buds bursting will be counted by the total number of buds on canes (COOMBE 1995; GILLIAN 1996; BENNETT 2002).

Shoot development: the primary bud of the compound bud gives rise to a shoot. The secondary and tertiary buds usually have less bud fruitfulness; however, they can produce shoots when the primary bud is dead (BENNETT 2002). The appearance of shoots and inflorescences are defined by E-L stages six to eleven which takes eight to ten weeks (COOMBE 1995).

Inflorescence development: E-L stages 12-18 define the formation of inflorescences as the shoots grow continuously. The development of flower parts takes 10-15 days since the fast formation and differentiation of flower of inflorescences primordia at grape growing stages 15. However, flowers will only be noticeable when shoots contain at least eight leaves (COOMBE 1995; BENNETT 2002).

Flowering: E-L stages 19-29 and is characterized by the formation of 16 leaves and nodes on the shoot. Phase 19 is known as the beginning of pollination. Full bloom occurs at 50% caps off and is finished at the phase of 26. Stages 27 shows fruit set when the berries begin to develop (COOMBE 1995). However, usually, only 20% - 30% of flowers can develop berries due to bad weather conditions thus, potential crop levels reduce.

Berry development: Enlargement of berries is described by E-L stages 31-34 and consists of three steps (1) the fast development of fruitlets into hard berries, (2) decrease in berry growth with initial of seed maturation, and (3) softening of berries followed by a change in color, that is known as veraison and is E-L stage 34 (COOMBE 1995; BENNETT 2002).

Harvesting: is comprised in E-L stages 38-47 and includes from harvest to end of leaf fail. Grapes are harvested when quality parameters such as color, sugar content, and acidity have reached desirable levels (COOMBE 1995; BENNETT 2002).

1.5 BUD FRUITFULNESS

In grapevine, there are two different buds named prompt and compound bud (SRINIVASAN AND MULLINS 1981). The prompt bud arises in the axil of the leaf and can burst in the same season's shoot (SRINIVASAN AND MULLINS 1981). The compound bud develops at the first node of the prompt shoot (summer lateral) and includes three different buds named primary, secondary, and tertiary buds (SRINIVASAN AND MULLINS 1981; VASCONCELOS *et al.* 2009). The primary bud produces leaf and flower primordia and under normal conditions and produces shoots in the next growing season (SRINIVASAN AND MULLINS 1981). The primary buds produce up to 4 inflorescence primordia depending on the variety (RAWNSLEY AND COLLINS 2005). Nonetheless, when primary buds are dead or damaged by environmental factors such as freezing, secondary buds can develop inflorescences primordia; however, the number of flower primordia will be smaller than found in the primary bud (LI-MALLET *et al.* 2015). The development of flowers in the grapevine is a two-year process and occurs in three distinctive stages (SRINIVASAN AND MULLINS 1981). The first stage occurs in the current growing season with the initiation of anlagen and its uncommitted primordia that can either develop into a tendril or

inflorescence primordia depending on environmental factors and physiological factors (GILLIAN 1996; CARMONA *et al.* 2008). After the anlagen primordia develop into floral development, the primordia branches repeatedly to produce a conical structure composed of many rounded branches to form the inflorescence primordia (SRINIVASAN AND MULLINS 1981). Each inflorescence primordia differentiates into individual flowers and this stage is called flower formation and occurs in the next growing season (SRINIVASAN AND MULLINS 1981; CARMONA *et al.* 2008). Yield in the grapevine is determined by the number of flower clusters per vine and the number of berries per cluster. Inflorescence development in the compound buds and the flower cluster development on the shoot defines potential bud fruitfulness and actual bud fruitfulness respectively (BENNETT 2002). Therefore, bud fruitfulness can be defined as the average number of clusters per shoot (DRY 2000; LI-MALLET *et al.* 2016). Although fruitfulness in the grapevine is a quantitative trait controlled by many genes, there are other factors that influence fruitfulness, such as climate, species, node position, and canopy management (DRY 2000; STRYDOM 2006).

1.6 BUD BREAK

Bud break is described as the first day when green tissue appears between the bud scales EL stage 5 (COOMBE 1995) and shoot emergence requires several weeks to complete. Delays in bud break and rate of growth can provide evidence of winter injury (FENNELL 2004). When the chilling requirement is fulfilled and temperatures rise to promote plant growth, bud break occurs (GARRIS *et al.* 2009). The dormant overwintering buds require low temperature, which is defined as chilling requirement, to transit from endodormancy to eco-dormancy (LAVEE AND MAY 1997; DOKOOZLIAN 1999; LONDO AND JOHNSON 2014). Chilling requirement in grapevine is between 7.2°C and 0°C (DOKOOZLIAN 1999;

LONDO AND JOHNSON 2014). When the environmental condition is favorable in terms of growth condition, for example, the temperature is higher than 15ºC, eco-dormancy is released (GARRIS *et al.* 2009). *Vitis vinifera* usually needs between 50 and 400 hours of chilling to satisfy endodormancy (LONDO AND JOHNSON 2014). When chilling hours increase (0 °C to 7.2°C), the rate of bud break increases varying by genotype (DOKOOZLIAN 1999; LONDO AND JOHNSON 2014). However, temperatures greater than 7°C lead to insufficient chilling thus, bud break is delayed and desynchronized (LONDO AND JOHNSON 2014). This results in low productivity in vineyards because it impacts the number of shoots and clusters as well as fruit ripening rates of vine (LAVEE AND MAY 1997; DOKOOZLIAN 1999).

1.7 QUANTITATIVE TRAIT LOCI (QTL) ANALYSIS

A QTL is a small segment of DNA on a chromosome responsible for a specific trait. QTL mapping is one of the most effective methods for providing genetic information underlying complex traits (COLLARD *et al.* 2005). Quantitative traits are measurable phenotypes resulting from the cumulative actions of many genes and the environment. Bud fruitfulness and bud break traits are known to be quantitative traits controlled by many genes with small additive effects. Thus, they cannot be studied using classical Mendelian genetics as the small effect of genes will be lost in background variation due to continuous variation across the population (FALCONER AND MACKAY 1996). QTL mapping, as a statistical approach, localizes chromosomal regions that highly affect the variation of quantitative traits in a population (ZENG 1994; MEKONNEN 2013; AWALE 2016). Construction of a linkage map using genetic markers that cover the whole genome of the organism and trait variation within a population is key in the QTL mapping (FANIZZA *et*

al. 2005; GRZESKOWIAK *et al.* 2013). The genetic mechanisms of complex traits are revealed by combining phenotype data, QTL analysis, and genome information to discover associated genes (ZENG 1994; FANIZZA *et al.* 2005; DHINGANI *et al.* 2015). Thus, QTL mapping is based on the principle of identifying a relationship between phenotype and the genotype of markers in a segregating population to explain the variation (recombination) in the trait of interest.

There are three commonly used QTL mapping methods: single marker analysis, simple interval mapping (SIM), and composite interval mapping (CIM) (JANSEN 1993; ZENG 1994; COLLARD *et al.* 2005; GRZESKOWIAK *et al.* 2013). The single-marker analysis does not need the existence of linkage map therefore, it lacks the power to detect QTL greater than 15 cM (centi-Morgan) away from the marker (COLLARD *et al.* 2005). To detect the significance of QTL in single-marker analysis, the t-test, linear regression, and analysis of variance (ANOVA) are used to detect QTL as statistical approach. Simple Interval Mapping (SIM) needs a linkage map as it utilizes the interval between two markers to locate a QTL, thus it is significantly more powerful than single-marker analysis. The logarithm of Odds (LOD) or Likelihood Ratio (LR) are used to test the significance of the QTL. Composite Interval Mapping (CIM) is similar to simple interval mapping, but it is more complex and superior. It uses the approach for discovering both linked and unlinked putative QTL positions thus, it is more reliable than other techniques (ZENG 1994; COLLARD *et al.* 2005). In CIM, a LOD score value that exceeds or equals a predicted value indicates a QTL position although the LOD score threshold depends on different factors from genome size and marker density (MANICHAIKUL *et al.* 2006). Moreover, the permutation test can identify the threshold of the maximum LOD score that can occur by

random chance. A permutation test shuffles genotypes and phenotypes by breaking the relationship between the two. A genome-wide maximum LOD on permuted data serves as the threshold as it characterizes the highest score produced by random chance. Due to technological developments, there are several software developed to run QTL analysis such as R/qtl, Map QTL, QTL-Cartographer which are most commonly used (JOEHANES AND NELSON 2008).

1.8 QTL MAPPING IN GRAPEVINES

Genetic mapping and QTL mapping are one of the most effective approaches for revealing genetic information underlying complex traits (COLLARD *et al.* 2005). QTL mapping identifies trait heritability, parent contribution and explains the percentage of variation in the trait. In grapevine, using QTL mapping with specific markers might aid in the selection of cultivars. There are several types of markers that have been used for QTL mapping over the last 20 years in the grapevine. The first genetic grapevine map published in 1995 was constructed by using random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) (LODHI *et al.* 1995; DALBÓ *et al.* 2000; ADAM-BLONDON *et al.* 2004; DOLIGEZ *et al.* 2006; WANG *et al.* 2012). Since these markers are dominant, it is difficult to transfer between populations (ADAM-BLONDON *et al.* 2004). Therefore, later studies focus on using simple sequence repeat (SSR) or single nucleotide polymorphisms (SNP) (WANG *et al.* 2012). Although SSR markers have some advantages over (AFLP) and (RAPD), such as a high level of polymorphism, they are expensive and time-consuming as they can require significant effort to develop (ADAM-BLONDON *et al.* 2004; TROGGIO *et al.* 2007). Single-nucleotide polymorphism (SNP)-based genetic markers received significant attention with the creation of a dense genetic linkage map for

grapevine in 2007 (TROGGIO *et al.* 2007). SNP is the most polymorphic and abundant marker and useful for identifying candidate genes for the trait associated with the QTL (RAFALSKI 2002; TROGGIO *et al.* 2007). The development of whole-genome sequencing and next-generation sequencing (NGS) technology accelerated the genetic map process (WANG *et al.* 2012). Moreover, the advent of genotyping by sequencing (GBS) which uses one of the reduced representation library (RRL) method has improved the genotyping cost per sample (ELSHIRE *et al.* 2011; HYMA *et al.* 2015). The first high density of SNP discovery using RRL in grapevine occurred in 2008 (BAIRD *et al.* 2008). GBS which is a theoretically simple and cost-effective method with high resolution is built on high throughput technologies (ELSHIRE *et al.* 2011; HYMA *et al.* 2015; YANG *et al.* 2016). This approach reduces the genome complexity and uses restriction enzymes to avoid the repetitive sequences of the genome (ELSHIRE *et al.* 2011; YANG *et al.* 2016). GBS was employed by BARBA et al. 2014 to reveal 16833 SNPs with an average density of 36 SNPs/Mbp to develop a map of grapevine. However, GBS markers have a high level of complexity and arbitrary sampling of sites and shallow sequencing strategy leads to missing data and genotyping error, and interpreting heterozygous as homozygous (HYMA *et al.* 2015; YANG *et al.* 2016). Although heterozygote under-calling has not been a problem with the application of GBS in inbred species, it is serious problem in heterozygous species such as grapevine (HYMA *et al.* 2015; YANG *et al.* 2016). This problem has been overcome in the grapevine by using computational approaches such as HettMapps which can produce a genetic map based on synteny with the reference genome (HYMA *et al.* 2015). Recently, RNase H2 enzyme-dependent amplicon sequencing (RhAmSeq) has been used to overcome the heterozygote under-calling experienced with GBS markers. The most

advanced grapevine genetic map consists of both GBS and RhAmpSeq markers that are highly informative across the *Vitis* genus.

Grapes are highly heterozygous and severely affected by inbreeding depression leading to poor seed viability and stunted growth (YANG *et al.* 2016; ZHOU *et al.* 2017). Thus, QTL mapping in grapevine, forest trees, and fruit crops usually exploits F1 mapping populations and pseudo-testcross strategy (WU *et al.* 2010). Nonetheless, an F2 population has been used to produce the genetic map and perform QTL analysis in grapevine (YANG *et al.* 2016; FENNELL *et al.* 2018). QTL mapping analysis has been performed for different traits in a grapevine F2 population to reveal the mechanism of genetics information of the traits (YANG *et al.* 2016). Specifically, in the past years, genetic analysis of grapevine has been focused substantially on disease resistance such as downy mildew, powdery mildew (FISCHER *et al.* 2004; WELTER *et al.* 2007; RIAZ *et al.* 2011; BARBA *et al.* 2014). Besides, QTL analysis related to grapevine agronomic traits such as berry size and weight, seed number, inflorescence number, flowering time, berry aroma profile, anthocyanin content and color, cluster architecture, number of clusters per vine, sexuality, sugar, and acid production, and pH and titrable acidity have also been conducted (DALBÓ *et al.* 2000; DOLIGEZ *et al.* 2002; FANIZZA *et al.* 2005; MARGUERIT *et al.* 2009; DOLIGEZ *et al.* 2010; DOLIGEZ *et al.* 2013; GRZESKOWIAK *et al.* 2013; VIANA *et al.* 2013; FECHTER *et al.* 2014; HYMA *et al.* 2015; ZHAO *et al.* 2015). So far, the QTL of many phenological traits of grapevine have been performed but study of the abiotic stress tolerances like drought and salt has been limited (BERT *et al.* 2013).

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CHAPTER 2: MAPPING OF QUANTITATIVE TRAIT LOCI CONTROLLING BUD FRUITFULNESS IN F2 GRAPEVINE POPULATION.

2.1 ABSTRACT

In grapevine, yield is determined by three different factors 1) the number of clusters per vine, 2) the number of berries per cluster, and 3) berry weight. The development of flower clusters differs with species, environmental conditions, and physiological factors. These all factors lead to different levels of fruitfulness. The number of potential clusters per bud is commonly referred to as bud fruitfulness. Thus, bud fruitfulness is one of the major and important traits for grapevine production. Identifying the genetics behind bud fruitfulness variation may aid to improve this trait to provide better grapevine cultivars with high productivity for future climate conditions. A segregating F_2 population developed by selfing a single hermaphroditic F_1 (16_9_2) derived from a cross between *V. riparia* and *Vitis* hybrid 'Seyval' was evaluated for bud fruitfulness in greenhouse conditions. The average number of fruitful buds for 10 node positions was used to evaluate bud fruitfulness. Exploratory data analysis showed that the number of flower cluster per node position varied across the F_2 population. F2 population and an integrated 2417 marker GBS- rhAmpSeq map was used to identify bud fruitfulness quantitative trait loci (QTL). Two bud fruitfulness QTL related to bud fruitfulness were identified on chromosome 4 and 5, explaining 16 % and 10% of the phenotypic variation, respectively. The additive effect showed that the male grandparent 'Seyval' is contributing positively to the average number of fruitful buds on the F2 genotypes

2.2 INTRODUCTION

Buds in *Vitis vinifera L.* are classified into two different categories namely the prompt bud, latent bud (SRINIVASAN AND MULLINS 1981). The first bud that arises in the axillary bud of the subtending leaf is described as the "prompt bud" and it can burst and form a shoot during the same season's shoot (SRINIVASAN AND MULLINS 1981). Nonetheless, sometimes this lateral shoot can be a vigorous fertile shoot if the growth of the lateral shoot is not inhibited by the main or primary shoot (BENNETT 2002).

The bud in the first prophyll of the prompt bud forms the overwintering buds known as compound bud or latent bud and it develops over the summer, forming six to nine node primordia and becomes endodormant at the end of the growing season. It contains three shoot meristems called primary, secondary, and tertiary buds (VASCONCELOS *et al.* 2009). The prompt bud develops into a lateral shoot in the same season. The primary bud forms in the axil of prophyll. The secondary and tertiary buds are initiated as the primary buds increases in size. In the primary bud, the leaf primordia develop first and then floral initiation may occur at the same time secondary and tertiary buds begin to develop (BENNETT 2002). These three buds are surrounded by bracts that protect them during dormancy in winter conditions (VASQUEZ AND FIDELIBUS 2006). Under normal conditions, the primary buds break and forms a shoot in spring and the other two buds do not break but remain viable. In the grapevine primary buds mostly contribute to flower clusters and it mainly produces between six and ten leaf primordia and up to four inflorescence primordia, depending on the variety. Nonetheless, if the primary bud is dead or damaged secondary bud may produce an inflorescence but the number of flower clusters and their size will be less than in primary bud (LI-MALLET *et al.* 2015).

In grapevines, there are three different stages in the formation of inflorescence that develops from lateral meristems (anlagen) in latent buds (SRINIVASAN AND MULLINS 1981; GILLIAN 1996). The first step is the initiation of anlagen (singular-anlage), it is initiated in the latent primary bud and it develops from June to dormancy. (SRINIVASAN AND MULLINS 1981). Anlagen are also known as uncommitted primordia and have a unique structure derived from shoot apical meristem or the axil of the leaf primordia of the primary bud (CARMONA *et al.* 2008; LI-MALLET *et al.* 2016). The anlagen can undergo repeated branching and generate inflorescence primordia, shoot primordia, or tendril primordia. Environmental and physiological conditions determine whether they develop into tendril or inflorescence primordia (GILLIAN 1996). For instance, cool, shady growing conditions are favorable for gibberellin production that promotes tendril formation. However, 10 °C soil temperature promotes cytokinin production in growing root tips and this stimulates the anlagen to develop into inflorescence primordia (GILLIAN 1996). Anlagen formation (also noted as an indicator or cluster axis formation) is the earliest indication of reproductive growth in the grapevine.

The second stage is inflorescence primordia formation and it is characterized by repeated branching during flowering (SRINIVASAN AND MULLINS 1981). The development of an inflorescence primordium continues as the anlagen start to divide and form branches (SRINIVASAN AND MULLINS 1981) and this occurs at the same time as the current growing season's crop set before harvest (GILLIAN 1996). Anlagen which generates two or more branches will produce tendrils, while anlagen which produce repeated branching will cause inflorescences. Inflorescence development continues in the following Spring. The conversion from inflorescence primordia to inflorescence starts after dormant buds are

activated in spring (WILLIAMS 2000; LI-MALLET *et al.* 2015). Branching of inflorescence primordia begins around 12 days before bud break and continues 12 to 15 days after the beginning of bud break until bloom (anthesis) (MAY 2000).

In grapevine, full mature latent buds containing one or more inflorescence primordia are described as fruitful buds, called bud fruitfulness (KHANDUJA AND BALASUBRAHMANYAM 1972; DRY 2000). Although grapevine yield is usually expressed as the weight of the harvested grapes per vine, bud fruitfulness is the most representative characteristic of yield (FANIZZA *et al.* 2005; LI-MALLET *et al.* 2015). Yield in the grapevine is determined by three different factors 1) the number of clusters per vine, 2) the number of berries per cluster and 3) berry weight. The number of inflorescences is accountable for up to 80% of the season-to-season yield variation (DIAS *et al.* 2019). Fruitfulness is determined inside of developing buds during the previous year and it may be assessed before pruning by review of a sample of buds, allowing growers to reduce fruit loss by leaving the best combination of spur length and spur or cane number. Bud fruitfulness (clusters per cane) can change by about 25% from year to year, and growers can get the first estimate of yield potential by dissecting buds and counting cluster primordia during the dormant season.

Not all buds develop inflorescence. Commonly, the first few nodes at the base of the cane tend to be less fruitful than nodes in the mid-cane region depending on the cultivar and species (SÁNCHEZ AND DOKOOZLIAN 2005). Bud fruitfulness increases from the base of the cane to the center and then decreases from the center towards the shoot apex (KHANDUJA AND BALASUBRAHMANYAM 1972). Central-Asiatic varieties such as Charas and Hussaine have their fruitful buds located farther from the base of the cane in contrast

to those of West-European origin such as Pinot, Riesling which is nearly as fruitful at the lower buds as in the middle of the cane (KHANDUJA AND BALASUBRAHMANYAM 1972). This is one of the reasons why some varieties are cane-pruned rather than spur-pruned. For example, *V.riparia* and 'Thompson Seedless' are known to have low bud fruitfulness in the first four nodes and so, they are cane-pruned (GERRATH AND POSLUSZNY 1988; GILLIAN 1996).

Many studies emphasize the effect of environmental conditions on inflorescence formation and potential bud fruitfulness (SÁNCHEZ AND DOKOOZLIAN 2005; CARMONA *et al.* 2008; LI-MALLET *et al.* 2015). Grapevines respond differently to varied temperature regimes and high temperatures promote inflorescence formation (LI-MALLET *et al.* 2016). There is a strong correlation between the temperature and the number of inflorescence appearing on the shoot during the inflorescence initiation of season 1 (SRINIVASAN AND MULLINS 1981). The temperature required for best induction and differentiation of inflorescence primordia differs with cultivar and geographical area (SRINIVASAN AND MULLINS 1981). For example, hybrid cultivars produce inflorescence flower clusters at 21 or 22°C, but *V. vinifera* cultivars need a temperature from 25 to 28°C (SRINIVASAN AND MULLINS 1981; LI-MALLET *et al.* 2015). Moreover, high temperatures promote the initiation of the second and third inflorescence, but low temperatures less than 20°C will promote the formation of tendrils in all varieties (VASCONCELOS *et al.* 2009). The maximum bud fruitfulness occurs with a temperature from 30°C to 35°C during inflorescence initiation.

Light quality and photoperiod affect the number of inflorescences, flower induction, and differentiation (LI-MALLET *et al.* 2016). Light intensity is the major

promoting factor for inflorescence induction and differentiation during the current growing season (LINKS 2014). The relationship between light intensity and fruitfulness of buds has been studied in the vineyard using different daylength exposure or canopy shading methods (SRINIVASAN AND MULLINS 1981). Exposure of the buds to sunlight increases fruitfulness, and shading or low light intensity reduces fruitfulness as the developing bud forms tendrils instead of clusters (SRINIVASAN AND MULLINS 1981). Sánchez and Dokoozlian 2005 revealed that that bud fruitfulness increases with increasing shoot light exposure. Shading for the first month after bloom can result in a reduction from 2.5 to 2.0 clusters per shoot (MARTINSON *et al.* 2012). Thus, when the buds are exposed to sunlight, bud fruitfulness increases as low light intensity reduces bud fruitfulness by encouraging tendril formation in developing buds (SÁNCHEZ AND DOKOOZLIAN 2005). Although environmental factors are important for bud fruitfulness, vineyard management watering and pruning also impact the number of flower clusters in grapevine. Water affects a wide range of plant processes such as photosynthesis and mineral nutrition and this has a direct effect on bud fruitfulness (LI-MALLET *et al.* 2015). Two characteristics of water status have important effects on the fruitfulness of latent buds during season 1: water deficit, and excessive water. Water stress reduces the fruitfulness of latent buds by affecting the number and size of inflorescence primordia (GUILPART *et al.* 2014).

The aim of pruning is to sustain a balance between vegetative and reproductive growth to ensure sufficient light exposure in the canopy and to develop a favorable structure for high crop production since shading affects bud fruitfulness. Although the pruning method depends on the cultivar and area, there are mainly two types of management cane and spur pruning (LINKS 2014). Since node position of fruitful buds

helps to determine pruning methods as cultivars such as *V.riparia* which tend to have more flower clusters in node position six to ten from the base of the cane required cane pruning (GUTIÉRREZ-GAMBOA *et al.* 2018) but those that have greater basal bud fruitfulness can be spur pruned (MARTINSON *et al.* 2012). As a result, the node position of bud fruitfulness and the number of inflorescences in the bud will determine the pruning methods that will be used with a particular grapevine cultivar

Another method of vineyard management is a training system which is one of the important factors as it may affect the bud fruitfulness since it develops the plant shape and sunlight exposure (LINKS 2014). Selecting the right training system will enable controlling vigor and vegetative growth so it can improve bud fruitfulness by providing better light interception during the growing season (CARTECHINI AND PALLIOTTI 1995; LINKS 2014). For example, vertical shoot positioning leads to shading of the lower buds, and this affects directly bud fruitfulness in the grapevine. Studies conducted on the Geneva Double Curtain training system show that bud burst and bud fruitfulness can be enhanced and yield improves by 44% when compared to other non-divided canopy methods (CARTECHINI AND PALLIOTTI 1995; LINKS 2014).

Environmental factors, vineyard management practices, genotype, and node position on bud fruitfulness are important for sustainable grape production. This leads to a hypothesis that genotype and node position on the grapevine affect bud fruitfulness. We expect to see some genotypes will be less fruitful in their basal nodes in the cane, some genotypes will present high fruitfulness in their middle of nodes and some genotypes will present fruitfulness across the whole cane. Moreover, the genotypes may segregate (1:2:1) in terms of the number of flower clusters per cane. Thus, the objectives of this study were

to determine the fruitfulness of bud according to node position and genotype effect on bud fruitfulness. The impact of environmental and physiological factors on the variation in the number of flower clusters has been widely investigated. Genetic studies on bud fruitfulness in grapevine have been shown that bud fruitfulness is a quantitative trait and controlled by many genes besides environmental effect. QTL associated with bud fruitfulness traits were found mainly on chromosome 3, 5, and 18 (FANIZZA *et al.* 2005; DOLIGEZ *et al.* 2010; GRZESKOWIAK *et al.* 2013). Thus, we hypothesized that chromosome 3, 5, and 18 are associated with bud fruitfulness and we expect to see QTL on these chromosomes. Therefore, another objective of this study was to improve knowledge on genetic determination of bud fruitfulness in interspecific hybrids for future use in marker-assisted selection (MAS) by locating some of the genomic regions involved in this trait.

2.3 MATERIALS AND METHODS

2.3.1 POPULATION DEVELOPMENT AND PLANT MATERIAL

F2 mapping population generated by selfing a single hermaphroditic F_1 (16_9_2) derived from a cross between *V. riparia* and 'Seyval' were used in the study (FENNELL *et al.* 2018). The parent F_1 and grandparents (*V. riparia* and Seyval), and 179 F2 progenies were clonally propagated and grown in the greenhouse at the South Dakota State University.

2.3.2 PLANT GROWTH AND MAINTENANCE

The F2 population, grandparents, and F_1 parent used in this experiment were established in 15-L pots. All vines were cycled annually through dormancy in a cold room at 4°C for at least 150 days. In spring, ecodormant grapevines were root pruned and repotted in 1:2:2 soil media (soil:peat: perlite by volume) to prevent root binding and to

maintain uniform growth through the studies. Vines were grown with a 25/20°C day/night temperature in climate-controlled greenhouses during May to August in Brookings, SD, USA (42°N lat). Vines were watered daily with a drip irrigation system and fertilized with 200 ppm nitrogen every two weeks. Vines were spur-pruned, leaving 2- 3 spurs per plant. After bud break, three or four shoots per plant were selected and trained vertically on bamboo stakes. Canes were collected by leaving the first node on the grapevines for future studies. Two canes that were representative of a typical dormant cane containing node 2 to 11 (2 = node closest to cane origin and 11 = apical node position) were collected from each F2 grapevines, grandparents, and F_1 parent in October 2019/20. Dormant canes were kept in the cooler to fulfill chilling requirement for two and a half months.

2.3.3 PHENOTYPIC ASSESSMENT

2.3.3.1. BUD BREAK PHENOTYPING

Dormant canes were sectioned into ten single node cuttings, tracking their node position from the original cane and placed in water trays in the laboratory, at 25°C to force. Bud fruitfulness was determined when the inflorescence emerged (stage E-L 18). Bud fruitfulness was evaluated as 1 or more flower clusters at a node position. Bud fruitfulness for each node and the total number of fruitful buds per cane were identified. Two canes were evaluated for each genotype and the average number of fruitful buds/cane was determined for each genotype.

The normality of bud fruitfulness distribution was evaluated by the Shapiro-Wilk test and histograms of frequency distribution were tested using R studio software. Data were normalized by applying the quantile normalization to the raw data at α= 5% for QTL analysis.

2.3.4 GENETIC INFORMATION AND QTL ANALYSIS

A genetic linkage map of 2417 GBS and rhAmpSeq markers and the average number of fruitful buds/ cane for each of 179 F2 progeny were used to determine bud fruitfulness QTL (Alahakoon et al. 2021, manuscript in preparation). Quantitative trait loci (QTL) was carried out using R software with the help of the R/qtl package (BROMAN *et al.* 2003). QTL analysis was performed using single QTL scan ("scanone" function, "Normal" model) and standard interval mapping (SIM) with R/qtl (the "scanone" function, map function "Kosambi", method="hk", n.perm=1000) and using F2 as the cross-type. Forward selection is used by SIM to identify the markers and the markers closest to each logarithm of the odds (LOD) peak. The significant threshold was determined by using permutation test (1000 times) at the alpha level of 0.05. All the QTL that crossed the LOD score of 3 (standard LOD threshold) were considered as a significant QTL. The QTL with the largest LOD was identified as the most possible QTL. Later the QTL was identified, the genotypic additive and dominance effects were conducted using ("fitqtl" function). The "bayesint" function was used to calculate for QTL and represented the region in which a QTL resides with probability ≥ 0.95. "Scan two" was used to detect the interaction between QTL. To determine node position fruitfulness QTL, data were converted to binary data according to the presence or absence of flower cluster on each node position, "YES", "NO" respectively. QTL mapping with binary data was performed using the binary model with R/qtl (the "scanone" function, map function "Kosambi", method=" binary", n. perm=1000) and using F2 as the cross-type. Effect plot and dot plot were produced by the function of "effectplot" and "plot.pxg" respectively.

2.4 RESULTS

2.4.1 PHENOTYPIC EVALUATION AND THE DISTRIBUTION OF BUD FRUITFULNESS TRAIT

The frequency distribution of bud fruitfulness was tested for one year. In the mapping population, the average number of fruitful buds per cane was 6.5 and bud fruitfulness ranged from 1 to 10 for each cane. Comparison of the parents with F2 genotypes showed that 'Seyval', male grandparent and F_1 had more fruitful buds/cane than that of the female grandparent, *V.riparia* (Figure 2-2).

The bud fruitfulness/cane was not normally distributed in the F2 population as indicated by the Shapiro-Wilks test with $\alpha = 5\%$. Bud fruitfulness values were highly skewed towards a large number of progenies having high bud fruitfulness/cane. The square root (sqrt) function was applied to the raw data but did not produce a normal distribution. Normality was achieved by applying the quantile normalization to the raw data at $\alpha = 5\%$ and with this data, the residuals of the regression model for QTL detection were normally distributed enabling QTL analysis with interval mapping.

2.4.2 QTL ANALYSIS USING AVERAGE BUD FRUITFULNESS/CANE AND BINARY BUD FRUITFULNESS AT EACH NODE POSITION

Two QTL for bud fruitfulness/cane were identified on chromosome 4 and 5 (Table 2-1). A QTL for bud fruitfulness observed on chromosome 4 explained 15.85 % of phenotypic variation with LOD score 7.06 at peak position 39 cM. The QTL region was ranged from 34.5151 cM to 48.014 cM in the genetic map. Another QTL associated with bud fruitfulness was detected on chromosome 5, explaining 9.71 % of phenotypic variation with LOD score 4.3 at peak position 43.79 cM. The QTL region ranged from 26.039 cM to 50.49 cM in the genetic map based on 95 % bayesian credible interval. The effect plot of two markers was used to indicate the nature of the interaction of these two QTL (Figure 2-6). According to the effect plot, the AA/BB group deviated a bit but it might be due to random variation as stepwiseqtl and scantwo results showed strong evidence that there was no significant interaction between two QTL. Additionally, the dot plot showed that genotypes with BB for both markers, rh_4_17799573 and GBS_5_18521423, had higher bud fruitfulness, and genotypes with AA for marker rh_4_17799573 and GBS_5_18521423, had lower bud fruitfulness (Figure 2-6). The genotype of the grandmother *V.riparia* for both marker was AA and the genotype of grandfather 'Seyval' was AB for GBS_5_18521423 and BB for rh_4_17799573.

Using a binary model, 10 QTL for bud fruitfulness in different node positions from 4 to 11 were located. Seven QTL related to different node positions (4^{th}, 6^{th}, 7^{th}, 8^{th}, 9^{th}, $10^{th,}$ and 11^{th}) were on chromosome 4. These seven QTL had similar peak positions and were located in the same region as the average percent bud fruitfulness/cane phenotype QTL on chromosome 4. Single QTL on chromosome 5 and chromosome 13 were found.

One of the binary QTLs on chromosome 5 was located with a similar peak position as the QTL identified using the average percent bud fruitfulness/cane phenotype (Table 2-2).

2.5 DISCUSSION

In our study, the average number of fruitful buds was used to evaluate bud fruitfulness in the F2 populationF2. Previous studies associated with bud fruitfulness among varieties showed that the difference in fruitfulness can be because of variation in cultivars and environmental factors (especially light and temperature), pruning and training method, even conditions in the previous growing season (SOMMER *et al.* 2000; GRZESKOWIAK *et al.* 2013). It seems that fruitfulness in this study was affected by cultivar and species characteristics types as parental and grandparent comparisons for QTL marker GBS_5_18521423 showed that Seyval (AB) contributed positively to bud fruitfulness in contrast to *V. riparia* (AA); however, the F2 genotype (BB) was more fruitful than both grandparents (Figure 2-4). Haplotype analysis for QTL on chromosome 4 also revealed that Seyval was BB for the peak marker and contributed to greater bud fruitfulness in comparison to *V.riparia* (AA) (Figure 2-5).

Node position on the cane can affect the fruitfulness as in some grapevine varieties, the first few nodes at the basement of the cane tend to be less fruitful than nodes in the middle of the cane (GUTIÉRREZ-GAMBOA *et al.* 2018). This is one of the reasons why spur-pruned and cane pruned are used in different varieties. For example, since the first two to three nodes are less fruitful in some varieties such as Concord and Thompson Seedless, they usually are cane pruned to (4-6 nodes) (GILLIAN 1996). In this study, node position affected the values of bud fruitfulness as the first four nodes (2 to 5) at the base of cane were less fruitful than nodes in mid-cane in this experiment (Figure 2-1). Node positions 7

to 9 had the greatest bud fruitfulness and node position 2 had the lowest bud fruitfulness (Figure 2-1). This low bud fruitfulness at the base of the cane could be balanced by long cane pruning.

Another factor affecting fruitfulness of base buds can be due to growing conditions of grapevines, for instance, compared to well-exposed shoots to the light, shoots exposed to shading are more likely to obtain nodes with less fruitful shoots during the next growing season (SRINIVASAN AND MULLINS 1981; DOLIGEZ *et al.* 2010; GRZESKOWIAK *et al.* 2013). Since our vines are grown in the greenhouse, the first four nodes may be affected by shading. The effect of shading can be reduced by doing shoot removal in the growing season and providing more light penetration to the grapevines. Thus, the genetic stability of bud fruitfulness is controversial as bud fruitfulness is affected by external factors (GRZESKOWIAK *et al.* 2013); however, Doligez *et al.* 2006 suggest that it can be stable despite differences in pruning method, environmental conditions, and different years.

Several QTL for bud fruitfulness in grapevine were previously identified on chromosomes 3, 5, 8, 14, and 18 (FANIZZA *et al.* 2005; DOLIGEZ *et al.* 2010; GRZESKOWIAK *et al.* 2013). In our study, two QTL on chromosome 4 and 5 for the average number of fruitful buds/canes were detected and explained a total of 25.56 % of the overall phenotypic variability (Table 2-1). Although a QTL on chromosome 5 was previously identified, the QTL on chromosome 4 differs from previous studies. This difference could be partly due to the segregation difference between crosses as the population in this study was an interspecific F2 population instead of a cross between two cultivars in the same species. It could also be a result of genotype x environment interaction or differences in trait measurement as (FANIZZA *et al.* 2005) measured the number of clusters per vine at harvest.

Here we measured the number of fruitful buds as flower clusters become visible after bud break. Similarly, Doligez *et al.* 2010 found a QTL on chromosome 5, which may be due to using a similar method for phenotyping, as he measured bud fruitfulness as the number of inflorescences per shoot at flowering time. The stability of these QTL in different years for bud fruitfulness has not been studied in this population and could vary with growing conditions.

In our study, we used fruitfulness of bud in different node positions, ranging from 2^{nd} to 11^{th} to check QTL with binary model and there were 9 QTL detected. Previous studies explained the importance of node position on bud fruitfulness in grapevine, but QTL analysis of fruitfulness by node position has not been studied yet. It could be due to the difficulty of tracking node position on vines since many studies were conducted on vines in field conditions. In our study, although most of the QTL associated with different node positions were on chromosome 4, there was two QTL on chromosome 5 and 13. Seven QTL found on chromosome 4 were co-located at the same position with node positions four through nine having the same bayesint confidence interval and peak marker.. This suggested that there can be one important candidate gene controlling bud fruitfulness. Based on our findings, node position had a significant genetic effect on bud fruitfulness (Table 2-2).

In this study, we compared two different methods, the number of fruitful bud/cane (SIM) and fruitfulness in different node positions (Binary), which showed similar results increasing confidence of the QTL on chromosome 4 representing bud fruitfulness.

2.6 CONCLUSION

Several components are contributing to yield in grapevine, but the yield is mainly correlated to the number of clusters per vine. Therefore, understanding the genetics associated with bud fruitfulness is crucial for increasing the productivity and sustainability of the grapevine. High heterozygosity and longtime generation in grapevine pose some difficulties in the genetic analysis of quantitative traits like bud fruitfulness. Our results confirmed that bud fruitfulness is a quantitatively inherited trait by controlling multiple gene loci. Bud fruitfulness of the F2 population showed variation and QTL analysis of the average number of fruitful buds allowed us to identify two QTL, one major QTL on chromosome 4 and one on chromosome 5. Another QTL analysis related to bud fruitfulness regarding node position revealed that with the "binary model" there were nine total QTL, and seven out of nine QTL were on chromosome 4 and there were two QTL, on chromosome 5 and 13 respectively. The QTL on chromosome 4 was co-located for almost all bud positions.

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2.8 TABLES AND FIGURES

Table 2-1: Bud fruitfulness QTL for the interspecific grapevine F2 population on chromosome 4 and 5.
The QTL were calculated in R/qtl using SIM, 1000 permutations, and p-value < 0.05.

Trait	Chr	LOD	Peak Position	Marker at the peak position	R^2 %	Genetic positon at 95% bayesint interval	Markers at the bayesint confidence interval	a (Trait unit)	d (Trait unit)
Bud Fruitfulness	4	7.06	38.95	rh_4_17799573	15.85	34.51511-48.01414	rh_4_14162870-rh_4_19762446	0.60	0.073
Bud Fruitfulness	5	4.3	43.79	GBS_5_18521423	9.71	26.03907-50.49815	GBS_5_6617972 - GBS_5_22425209	0.48	0.005

Chr, chromosome, LOD, likelihood of odds, R2, phenotypic variation explained by this QTL, a, estimated additive effect (trait unit), and d, estimated dominance effect.

Table 2-2: Binary bud fruitfulness QTL for cane node position on chromosome 4, 5, and 13.
QTL for node position (2 to 11) using the binary model in R/qtl using Binary model, 1000 permutations.

Trait	Chr.	LOD Score	Peak Position (cM)	Marker at the peak position	Genetic position at 95% bayesint interval	Markers at the bayesint confidence interval
BF_Node 11	4	3.91	56.9	rh_4_22127549	29.23108-63.89218	rh_4_7614182-GBS_4_24606925
BF_Node 10	4	4.094	31.47	rh_4_9681702	16.279-63.89236	GBS_4_3284361-GBS_4_24606925
BF_Node 9	4	5.39	61	GBS_4_23839694	51.25143-63.89254	GBS_4_20482368-GBS_4_24606925
BF_Node 8	4	5.37	57.6	rh_4_22331368	51.25143-63.89254	GBS_4_20482368-GBS_4_24606925
BF_Node 7	4	5.58	58.9	GBS_4_22856230	51.83944-63.89254	GBS_4_20619675-GBS_4_24606925
BF_Node 6	4	5.57	58.9	GBS_4_22856230	51.83944-63.89254	GBS_4_20619675-GBS_4_24606925
BF_Node 4	4	5.44	52.1	rh_4_20739437	42.004-62.908	rh_4_18311071-GBS_4_24561501
BF_Node 10	5	4.09	54.1	rh_5_23735988	15.419-60.30737	rh_5_3959240-GBS_5_25438857
BF_Node 10	13	4.04	22.3	GBS_13_5623472	16.372-24.767	GBS_13_4338318-rh_5_6351187

BF, bud fruitfulness, Chr, chromosome, LOD, likelihood of odds, R2, phenotypic variation explained by this QTL.

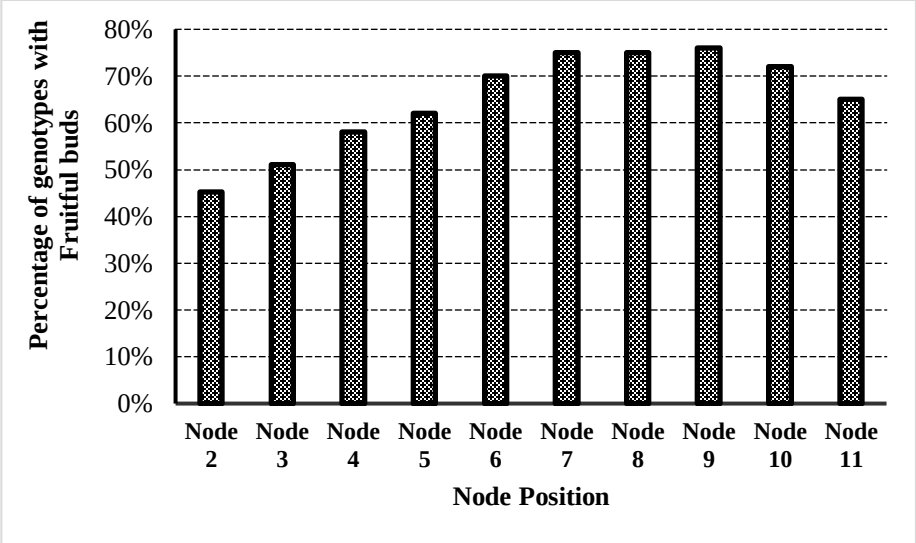

Figure 2-1: Percent fruitful buds by node position.
The x-axis represents the node position (2 to 11) and the y-axis represents is the average percentage of genotypes with fruitful buds (n=2 canes for each of 179 genotypes).

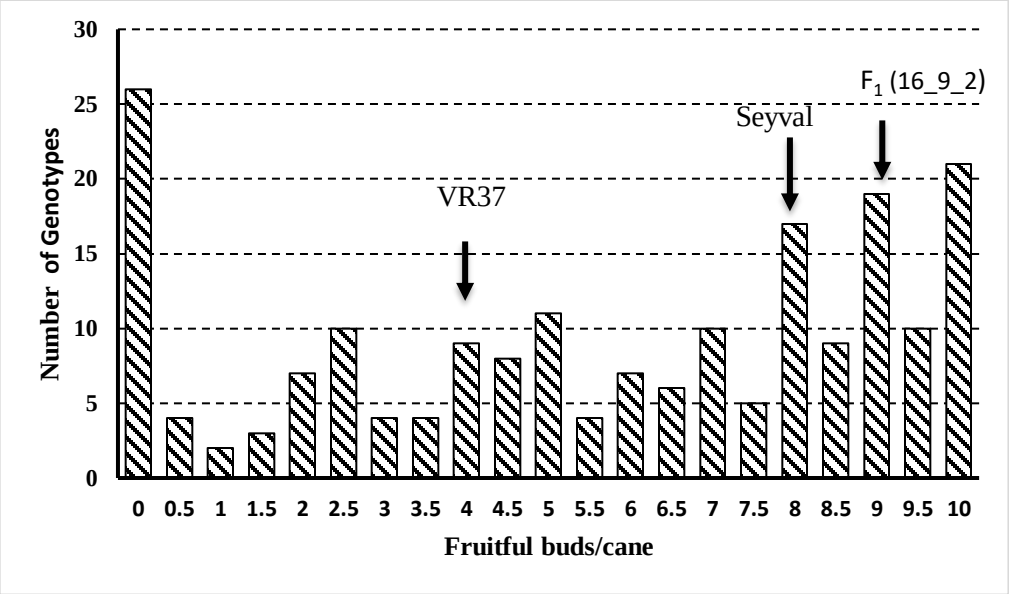

Figure 2-2: The frequency distribution of the average number of fruitful buds per cane in the F2 population.

The x-axis is the average number of fruitful buds per cane and the y-axis is the number of genotypes in each class. The parent and grandparent phenotypes indicated by arrows (F_1 (16_9_2), V. riparia (VR37), and ‘Seyval’)

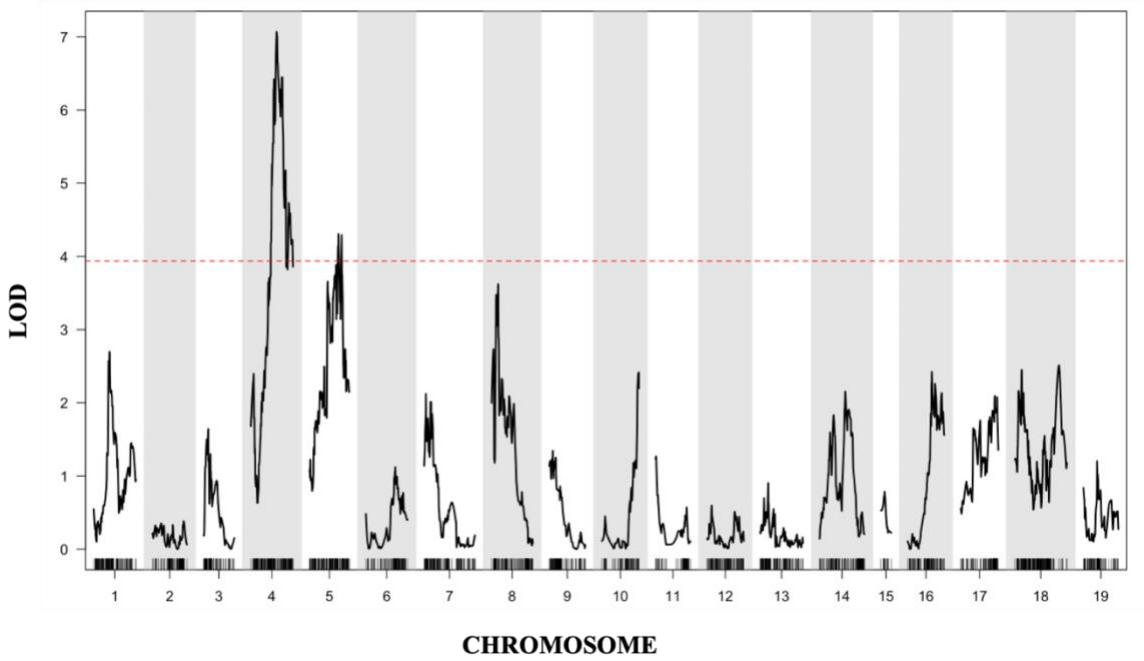

Figure 2-3: Bud fruitfulness QTL on chromosome 4 and 5.
QTL were calculated using SIM in R/qtl, 1000 permutations, and a significant p-value < 0.05.

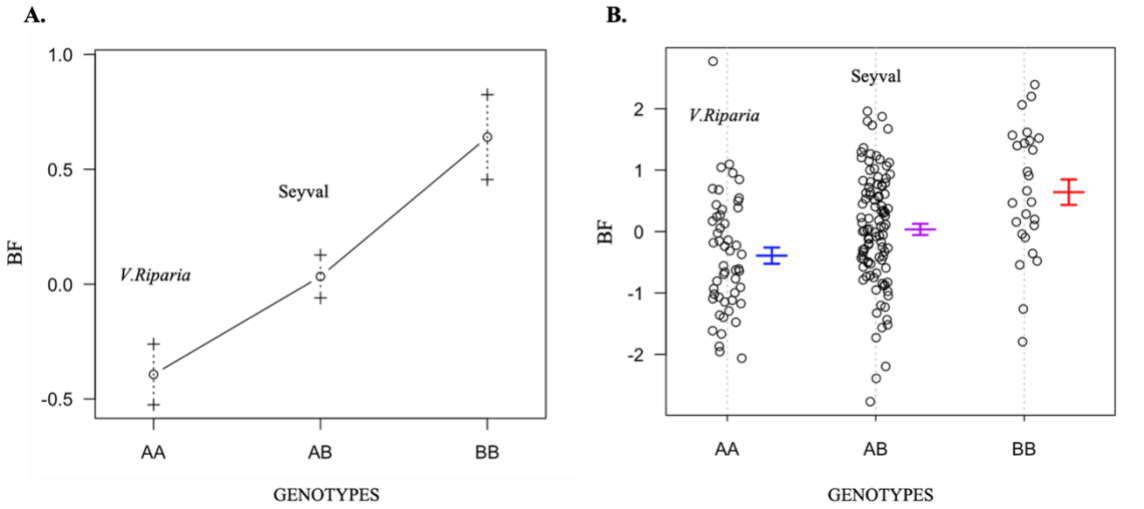

Figure 2-4: Genotype effect and dot plot for average bud fruitfulness (BF) QTL peak marker on chromosome 5.

Genotype frequency distribution for peak marker GBS_5_18521423 for QTL on chromosome 5. Genotype AA represents *V. riparia* female grandparent. Genotype AB represents 'Seyval' male grandparent. Values are the normalized average bud fruitfulness. A. Genotype effect plot of the normalized average bud fruitfulness for marker GBS_5_18521423. B. Distribution of the normalized average bud fruitfulness for marker GBS_5_18521423.

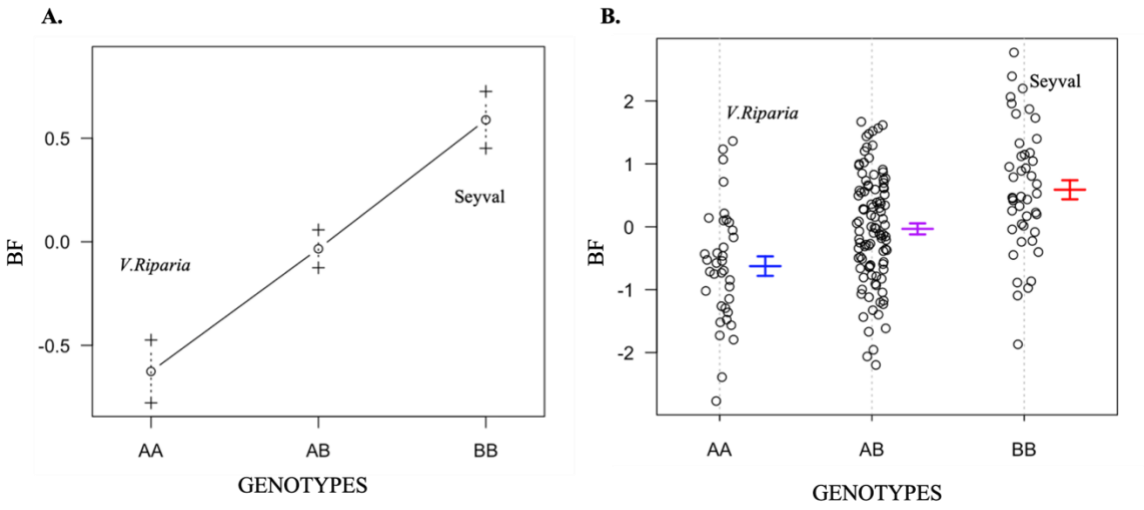

Figure 2-5: Genotype effect and dot plot for average bud fruitfulness (BF) QTL peak marker on chromosome 4.

Genotype frequency distribution for peak marker rh_4_17799573 for QTL chromosome 4. Genotype AA represents *V. riparia* female grandparent. Genotype BB represents 'Seyval' male grandparent. Values are the normalized average bud fruitfulness. A. Genotype effect plot of the normalized average bud fruitfulness for marker rh_4_17799573. B. Distribution of the normalized average bud fruitfulness for marker rh_4_17799573.

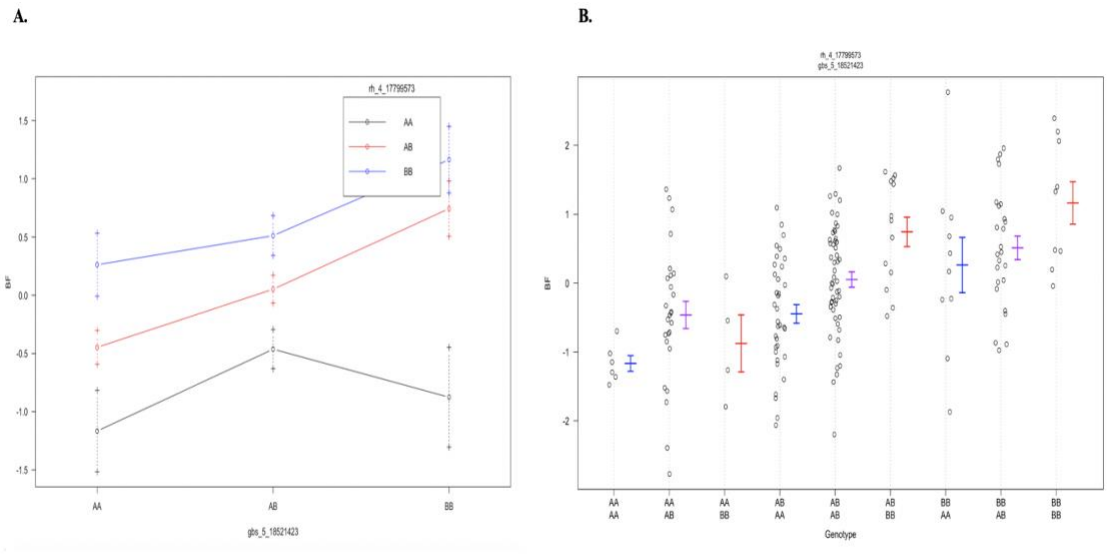

Figure 2-6: Genotype effect of two QTL on chromosome 4 at marker rh_4_17799573 and 5 at marker GBS_5_18521423 for average bud fruitfulness (BF).
A: Estimated phenotype averages for each of the three-locus genotype groups for both marker GBS_5_18521423 and Rh_4_17799573. Values are the normalized average bud fruitfulness. Genotype AA represents *V. riparia* female grandparent. Genotype AB represents 'Seyval' male grandparent for GBS_5_18521423. Genotype AA represents *V. riparia* female grandparent and genotype BB represents 'Seyval' male grandparent for Rh_4_17799573. B: A dot plot of the phenotype as a function of marker genotypes, first row is Rh_4_17799573 and second row is GBS_5_18521423 in the x-axis. Grandparent *V. riparia* is genotype AA and 'Seyval' is genotype BB for peak marker rh_4_17799573. Grandparent *V. riparia* is genotype AA and 'Seyval' is genotype AB for peak marker GBS_5_18521423.Values are the normalized average bud fruitfulness.

CHAPTER 3: MAPPING OF QUANTITATIVE TRAIT LOCI CONTROLLING BUD BREAK IN F2 GRAPEVINE POPULATION.

3.1 ABSTRACT

The grapevine developmental stages can be defined by three main phenological stages: 1) bud break, 2) flowering 3) veraison. Bud break determines the vegetative growth of vines and has a great impact on the fruiting of the next production season. Therefore, both early and late bud break can damage grapevines and decrease grape production. Thus, in this study, we aimed to determine the genetic character of the bud break rate in fully chilled buds to identify the genetic basis of the trait and identify individuals with delayed bud break character for future crosses. An F2 mapping population developed by selfing a single F_1 plant derived from a cross between *Vitis riparia* and the *Vitis* hybrid wine cultivar 'Seyval' was used with a population subset of 179 genotypes to monitor bud break in chilling fulfilled canes. Bud break growth stages were evaluated for one month according to the modified Eichhorn and Lorenz (modified E-L) scale for each genotype with two different budburst methods. The concept of the area under bud break curve (AUBPC) for bud development for one month, bud break score at the third week, and bud break rate were used to analyze bud break. Exploratory data analysis revealed that AUBPC varied within the population and had a significant difference among the node positions. QTL mapping was conducted using an integrated GBS and rhAmpSeq map (2519 markers) using standard interval mapping (SIM). One QTL related to bud break, explaining 11.04 % phenotypic variation was identified on chromosome 10. This marker associated with the QTL region can be used to identify genotypes and select new cultivars suitable for changing climate conditions.

3.2 INTRODUCTION

In perennial plants, including grapevine, endodormancy is triggered by decreasing day length and temperatures at the end of the growing season (FENNELL 2004; ROHDE AND BHALERAO 2007). In this dormancy phase, grapevine bud will not break and resume growth even under favorable conditions (LAVEE AND MAY 1997). During the endodormancy phase, the chilling requirement of the grapevine needs to be fulfilled to transition the vine to ecodormancy when the ecodormant bud can burst and continue to grow in the next growing season (DOKOOZLIAN 1999). The chilling requirement in grapevine is fulfilled by species and cultivar-specific chilling hour (0ºC to 7.0ºC) accumulation. The genotype-specific chilling hour accumulation for transitioning from endodormancy to ecodormancy varies from 50-400 hours (0ºC to 7.0ºC) while other species range between 250-2250 hours *(DOKOOZLIAN 1999; LONDO AND JOHNSON 2014)*. The chilling requirement is crucial to protect vines from breaking bud too early in response to short intervals of warm temperatures followed by cold temperatures.

Bud break is described as the first appearance of green tissue through the bud scales or the emergence of a new shoot from a bud during the spring (COOMBE 1995; LORENZ *et al.* 1995; COOMBE AND DRY 2004). Therefore, understanding the genetic mechanism behind bud break helps to select and develop new varieties according to changing environmental conditions.

Bud break in perennial plants is under genetic control, quantitative in nature, and controlled by multiple genes along with environmental effect (ROHDE AND BHALERAO 2007; OLUKOLU *et al.* 2009; FAN *et al.* 2010; ROHDE *et al.* 2011; FABBRINI *et al.* 2012; ALLARD *et al.* 2016). QTL analysis has also been performed in several perennial species

such as apricot, peach, pear, and apple (OLUKOLU *et al.* 2009; FAN *et al.* 2010; GABAY *et al.* 2018). These studies reveal that bud break in perennial fruit crops is a complex genetic trait controlled by several strong QTL and many small contributing QTL. Although bud break in grapevine has been studied, only a few studies have focused on the genetic of this trait (LONDO AND JOHNSON 2014; FENNELL *et al.* 2018).

An understanding of the genetic determination of bud break is crucial to select grapevine materials for cold winter regions in a changing climate to promote grapevine and grape production sustainability. This study phenotyped the rate of bud break in fully chilled buds from an F2 population of grapevine and used quantitative trait loci analysis in two different bud break studies to investigate the genetic mechanism responsible for this trait.

3.3 MATERIALS AND METHOD

3.3.1 PLANT MATERIAL AND POPULATION DEVELOPMENT

The F2 mapping population which comprised of 179 individuals was developed by selfing a single hermaphrodite F_1 (16_9_2) from the cross between *V. riparia* and 'Seyval' (GARRIS *et al.* 2009). The *V. riparia* and 'Seyval' grandparents of F_1 and F2 progenies were clonally propagated and evaluated under greenhouse conditions in South Dakota State University, Brookings, South Dakota. The *V. riparia* grandparent is identified to be early bud break while 'Seyval' grandparent shows slower bud break.

3.3.2 Growth and maintenance of grapevines

Grapevines used in this experiment were established in 15-L pots and cycled annually from endodormancy to ecodormancy in a cold room at 4 °C for at least 150 days. In spring, ecodormant spur pruned vines were root pruned and repotted in 1:2:2 soil media (soil:peat: perlite by volume) to maintain uniform growth through the studies. vines were

grown with a 25/20°C day/night temperature in climate-controlled greenhouses during May to August in Brookings, SD, USA (42°N lat). Vines were watered daily and fertilized with 200 ppm nitrogen every two weeks. After bud break, three or four shoots per plant were selected and trained vertically on bamboo stakes. From October to November after grapevines entered endodormancy, two canes were collected from each of 179 F2 genotypes, parent (16-9-2) and grandparents (*V. riparia* and Seyval), and placed at 4°C for chilling fulfillment.

3.3.2.1 FORCING BUD BREAK IN THE LABORATORY (EXPERIMENT 1)

Canes containing 10 nodes (node 2 to 11) were kept in cooler to fulfill chilling requirement for around two and a half months. After that, the ecodormant canes of 79 genotypes were sectioned into single-node cuttings from the base (node 2) to apical position (node 11) (maintaining node identify) and each cutting was placed into water trays in the laboratory under 24 photoperiod and 25/20 ºC. Water was added to forcing containers daily so that the water level stayed just below the bud. Each day, the growth stage of the bud was recorded according to the modified Eichhorn-Lorenz bud phenology scale (modified E-L scale) (COOMBE 1995). Bud break was monitored for consecutive 25 days (Figure 3-4). After that, the buds that did not show any swelling were cut in half to see if the meristems were alive or dead. Browning of the bud tissue indicated that the bud is dead and these nodes were removed from the data set.

3.3.3.2 FORCING BUDS IN GREENHOUSE WITH ROOTING MEDIA (EXPERIMENT 2)

After vines enter dormancy, 12 node canes for each genotype were taken from the greenhouse and sectioned into cuttings containing 2-3 buds. Each cutting was left into the

cooler to fulfill the chilling requirement for around three months. Each cutting was taken out from the cooler and placed in water for 3 or 4 days to rehydrate before planting. Six single node cuttings for each genotype were planted in germination media (Pro-mix, Sunshine germination media) in 13h photoperiod and 25/20 ºC, and cuttings were watered regularly. Each day for one month, the growth stage of buds was measured according to the modified Eichhorn-Lorenz bud phenology scale (modified E-L scale) (EICHHORN AND LORENZ 1977; COOMBE 1995).

3.3.3 MODIFIED E-L BUD SCORING

Bud break in grapevine is evaluated using the modified E-L system for growth stages to score the phenological stage (EICHHORN AND LORENZ 1977; COOMBE 1995). E-L number score 2-3 (2 for bud swell and 3 for wooly bud) and bud break is identified by green tip or first leaf visible (stage 4). E-L score 5 to 11 belongs to shoot elongation in the modified E-L system for grapevine growth. All stages in shoot development including E-L score 7 (the first leaf separated), 9 for 2-3 leaves separated, and 11 for 4 leaves separated are crucial stages for measuring the bud elongation process. The time need to reach a particular developmental stageis dependent on the genotype and environmental conditions (COOMBE 1995)

3.3.4 AREA UNDER BUD BREAK PROGRESSION CURVE (AUBPC) AND STATISTICAL ANALYSIS

Area under curve (AUC) concept was applied in our bud break data as disease intensity and phenological stage of bud development show a similar tendency, ie that the disease intensity and phenological stages of bud development increase with time (JEGER

AND VILJANEN-ROLLINSON 2011). To illustrate, if appropriate conditions are provided, the bud in stage 4 in week 2 may be in stage 5 next week. The area under the bud break progression curve (AUBPC) provides numerical information on the rate of bud break and phenological development. The AUBPC was calculated in R studio using the function auc from the MESS library for each experiment. The calculated AUBPC value was used to perform QTL analysis. All phenotypic evaluation analyses including the distribution of data with histogram and normality of data checked with Shapiro-Wilk test was performed in RStudio. The node positions affect on AUBPC was analyzed by ANOVA.

3.3.4 QUANTITATIVE TRAIT LOCI ANALYSIS

Quantitative trait loci (QTL) analysis was performed using average AUBPC value for both data sets consisting of 79 genotypes in the lab and 179 genotypes in the greenhouse, respectively, and integrated GBS and rhampSeq linkage map (2519 markers) with the R/qtl package (BROMAN *et al.* 2003). QTL analysis was performed for both AUBPC data sets using single QTL scan ("scanone" function, "Normal" model) and standard interval mapping (SIM) with R/qtl (the "scanone" function, map function "Kosambi", method="hk", n. perm=1000) and using F_2 as the cross-type. The significance threshold was determined with 1000 permutations and a p-value < 0.05 and grapevine standards of 1000 permutations. The QTL identified were then fitted in a model, "AUC~QTL+e" to obtain the genotypic additive and dominance effects ("fitqtl" function). Confidence intervals were calculated as Bayesian credible intervals (bayesesint) with a probability of coverage of 0.95.

3.4 RESULTS

3.4.1 PHENOTYPIC EVALUATION

The AUBPC (area under the bud break progression curve) was calculated in R studio and varied with F2 genotypes, parent (16- 9-2) and grandparents. Phenotypic data of the F2 population was normal, therefore no transformations were applied to the variables. The AUBPC values for each node position were compared to reveal the node position effect on bud break. The buds closer to the base of the cane (node position 2, 3, and 4) tended to break early; consequently, they have a greater AUBPC value. The more distal buds near the cane apice (9, 10, and 11) tended to break more slowly (Figure 3-1). AUBPC values ranged from 90 to 160 units with the majority of genotypes falling between 130 and 140 AUBPC. The node position effect was significant based on $P < 0.001$.

3.4.2 QTL ANALYSIS OF AUBPC RESULTS

There was one minor possible QTL controlling bud break, but this QTL was not significant as the peaks did not cross the threshold of 1000 permutations for the lab and water-based assay (79 genotypes). However, the QTL on chromosome 12 at peak position 26.4 cM was exceeding 10 % of the threshold value (Table 3-1). In the greenhouse and soil bud break assay, a single QTL for AUBPC was identified on chromosome 10 at peak position 44.30 with LOD score of 4.55 (Table 3-2). The genetic effect plot of the nearest marker to QTL peak indicated that early bud break is associated with *V. riparia* grandparent (AA) and the slower bud break is derived from the heterozygote grandparent ‘Seyval’ (AB) for marker GBS_10_10801568. The homozygous BB genotypes had the lowest AUBPC and therefore the slowest bud break and shoot development (Figure 3-3).

3.4.3 QTL ANALYSIS OF BUD BREAK SCORE AND BUD BREAK RATE RESULTS

QTL analysis was performed separately for bud break E-L score at third week and the bud break rate (Week4 -Week2) in soil-based bud break assay with transformed values. QTL information for these two traits including LOD score, peak position marker, positions and flanking markers at 95% interval, percentage variation, and additive and dominance effects are provided in Tables 3-3 and 3-4. All three measures of bud break phenotype (AUBPC, E-L score at week 3, and bud break rate (E-L score for Week 4-Week 2) showed similar QTL on chromosome 10 with the same peak marker. However, AUBPC QTL explained 11% of the phenotypic variation instead of 9% and had the smaller bayesint confidence interval.

3.5 DISCUSSIONS

Selection for grapevine adaptability to changing climate conditions requires a better understanding of the genetic mechanism controlling grapevine growth initiation in the spring. The majority of the QTL analysis in grapevine has been conducted on flowering and berry ripening timing in large vines under field conditions (COSTANTINI *et al.* 2008; FECHTER *et al.* 2014). Additionally, bud break is a genetically controlled trait and several bud break by chilling fulfillment QTL mapping studies have been done to reveal the genetic mechanism of bud break in many species.

This study used an F2 population derived from grandparents with fast and slow bud break characteristics. Besides, the study was performed under controlled conditions using single node sections to sustain uniform environmental conditions and improve the ability to

identify genetic control and to detect candidate markers. Bud break is a heritable complex quantitative trait controlled by many genes (FENNELL AND HOOVER 1991). Both early and late bud break can damage vines as early bud break may increase the vulnerability of vines in cold climates (LAVEE AND MAY 1997). However, late bud break may have an impact on the maturity and productivity of grapevines. Thus, QTL mapping of this trait was conducted to identify marker-trait associations in bud break. Breeding and QTL mapping in woody fruit crops like grapevine typically use a small population size (80-100 progeny) due to long generation time and large space for maintenance of grapevines (YANG *et al.* 2016). This study used 179 (greenhouse experiment) and 79 (laboratory experiment) progeny and one year's data. In our greenhouse experiment, we tested three measurements of bud break (E-L bud break score at the third week, the concept of area under bud break progressive curve (AUBPC), and bud break rate (E-L score for week 4 – E-L score for week 2) (DUAN *et al.* 2012; LONDO AND JOHNSON 2014). These phenotypes successfully explained three different aspects of bud break in grapevine as we measured bud break through time (AUBPC) with repeat measures and genotypes at specific time points to address bud break dynamics. E-L bud break score at third week showed the highest variation across genotypes for any of the time points monitored. AUBPC consolidated many repeat measurements into one value for QTL analysis and within AUBPC, all the processes starting from the initiation of bud break to shoot emergence and elongation are included. Integration of these three methods in our study helped to maximize the data usage, extract all valuable information as well as increased our confidence for major QTL on chromosome 10. Additionally, with the presence of A allele, *V. riparia* grandparent

contributes to early bud break and ‘Seyval’ grandparent contributes to relatively slow bud break due to the presence of B allele.

In our second experiment performed in the lab, we identified one minor QTL using AUBPC which has not been reported previously. Comparing two different methods of bud forcing revealed one major QTL on chromosome 10 and one minor QTL on chromosome 12.

The QTL affecting the berry and phenology-related traits have been found on chromosome 18 (DOLIGEZ *et al.* 2002; COSTANTINI *et al.* 2008); however, QTL associated with phenological traits such as flowering, berry set, and ripening have been also discovered on chromosome 1,6,7,8,12,15, and 16 in field-grown vines (DOLIGEZ *et al.* 2002; COSTANTINI *et al.* 2008; GRZESKOWIAK *et al.* 2013; FECHTER *et al.* 2014). QTL for budburst have recently been detected on chromosome 4, 15, and 19 in different *V. vinifera* field-grown populations (DUCHÊNE *et al.* 2012; GRZESKOWIAK *et al.* 2013). Recently, a QTL identified for the rate of bud break in greenhouse-grown grapevines in contrast to cuttings used in this study was confirmed on chromosome 19 by (FENNELL *et al.* 2018). In this study using single node cuttings, a new QTL for bud break value (AUBPC), bud break rate, and bud break E-L score in the greenhouse was identified on chromosome 10 at peak position 44.3. This represents the first bud break QTL detected in grapevine on chromosome 10 associated with bud break and may be influenced by using separate nodes removed from cane apical dominance effects. 1) Previous studies have been conducted in intact grapevines in a field or greenhouse. 2) The method of bud break data collection in previous studies was to determine the date 50 % of buds on the grapevine reached the required EL stage 5, which is “visible green tip” (GRZESKOWIAK *et al.* 2013). In this study, the AUBPC value captured both the timing of break and the rate of phenological

development instead of identifying days to the specific stage of development. 3) Canes used in this study were fully chilled and bud break was measured under constantly controlled conditions instead of variable field conditions. Thus, it is very clear that bud break can differ between genetic background and environmental conditions. Besides, this study measured the full duration of the bud break process and provides a protocol for continued to study of the genetic control of the rate of bud break and phenological development.

3.6 CONCLUSION

Adapting grapevine cultivars to future climatic conditions is a main challenge for the future and bud break is one of the most important phenological data considered in grapevine breeding and sustainability. Selection for early or late phenological bud break stages depends on regional conditions. For example, late bud burst is preferred in regions with an increased risk for spring frost; however, early bud burst is preferred in grape-growing areas with continental influenced climate conditions and short growing seasons. Understanding of genetic mechanisms of bud break will help not only increase grape production sustainability by developing viticultural strategies for spring freeze mitigation but also provide information for the development of vines for regions with spring freeze risk or short growing seasons. In this study, a QTL with SIM was detected on chromosome 10, contributing 11% of the phenotypic variation. This result can be used in providing more information to help to identify genes associated with bud break and produce markers for marker-assisted selection to generate suitable cultivars for cold environments.

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3.7 TABLES AND FIGURES

Table 3-1: AUBPC QTL on chromosome 12 for the F2 population.
QTL were calculated in R/qtl using SIM, 79 genotypes, 1000 permutations, and p-value < 0.010. The QTL had 3.0 above LOD score in the table.

Trait	Chr.	LOD Scores	Peak Position (cM)	Nearest Marker	R^2 %	Genetic position at 95% bayesint interval	Markers at the bayesint confidence interval	a (Trait unit)	d
AUBPC	12	3.5	26.4	rh_12_6691567	18.46	2.3521-4.535006	rh_12_1089461-GBS_12_9587137	-4.49	12.13

AUBPC, area under bud break progression curve, Chr, chromosome, LOD, likelihood of odds, R2, phenotypic variation explained by this QTL, a, estimated additive effect, and d, estimated dominance effect.

Table 3-2: AUBPC QTL on chromosome 10 for the F2 population.
QTL were calculated in R/qtl using SIM, 179 genotypes, 1000 permutations, and p-value < 0.05.

Trait	Chr	LOD	Peak Position (cM)	Marker at peak position	R^2 %	Genetic position at 95% bayesint interval	Markers at the bayesint confidence interval	a (Trait unit)	d (Trait unit)
AUBPC	10	4.55	44.3	GBS_10_10801568	11	26.80703-47.06007	rh_10_5247875-rh_10_12409898	-13.06	-0.82

AUBPC, area under bud break progression curve, Chr, chromosome, LOD, likelihood of odds, R2, phenotypic variation explained by this QTL, a, estimated additive effect, and d, estimated dominance effect.

Table 3-3: Week three bud break Score QTL on chromosome 10 for F2 population.

QTL were calculated in R/qtl using SIM, 179 genotypes, 1000 permutations, and p-value < 0.010.

Trait	Chr	LOD	Peak Position (cM)	Marker at peak position	R^2 %	Genetic position at 95% bayesint interval	Markers at the bayesint confidence interval	a (Trait unit)	d (Trait unit)
E-L score*	10	3.75	44.3	GBS_10_10801568	9	26.066-56.03	GBS_10_4583458-GBS_10_22647189	-4.198	-0.006

*E-L score at 3 weeks, Chr, chromosome, LOD, likelihood of odds, R2, phenotypic variation explained by this QTL, a, estimated additive effect and d, estimated dominance effect.

Table 3-4: Bud break Rate QTL on chromosome 10 for F2 population.
QTL were calculated in R/qtl using SIM, 179 genotypes, 1000 permutations and pvalue < 0.010.

Trait	Chr	LOD	Peak Position (cM)	Marker at peak position	R^2 %	Genetic position at 95% bayesint interval	Markers at the bayesint confidence interval	a (Trait unit)	d (Trait unit)
Bud Break Rate*	10	3.75	44.3	GBS_10_10801568	9	26.066-56.03	GBS_10_4583458-GBS_10_22647189	-4.198	-0.006

*Bud break rate (E-L score at week 4 – E-L score at week 2), Chr, chromosome, LOD, likelihood of odds, R2, phenotypic variation explained by this QTL, a, estimated additive effect, and d, estimated dominance effect.

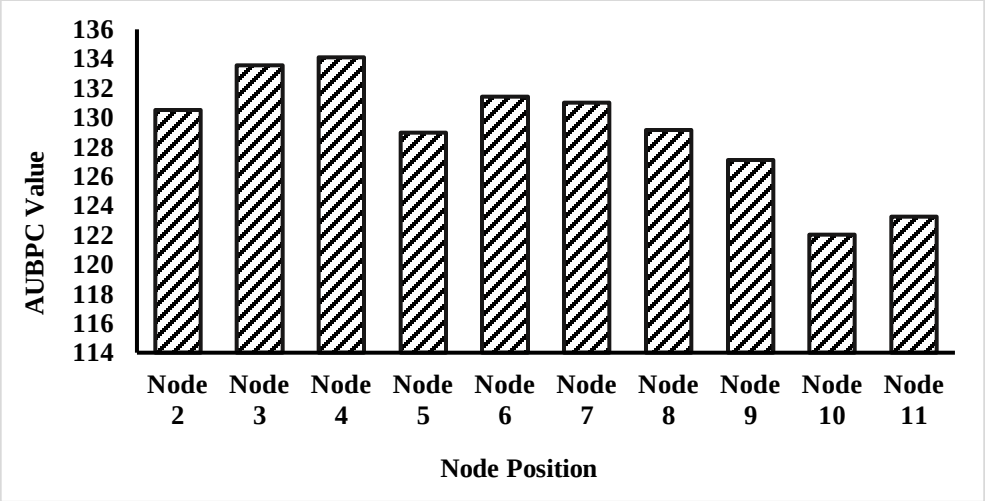

Figure 3-1:Area under bud break progression curve (AUBPC) influenced by node position. The x-axis is cane node position and the y-axis is the average AUBPC value for each node position in the laboratory water-based bud break assay (n=2, 79 genotypes, a one-way ANOVA, and p-value < 0.001).

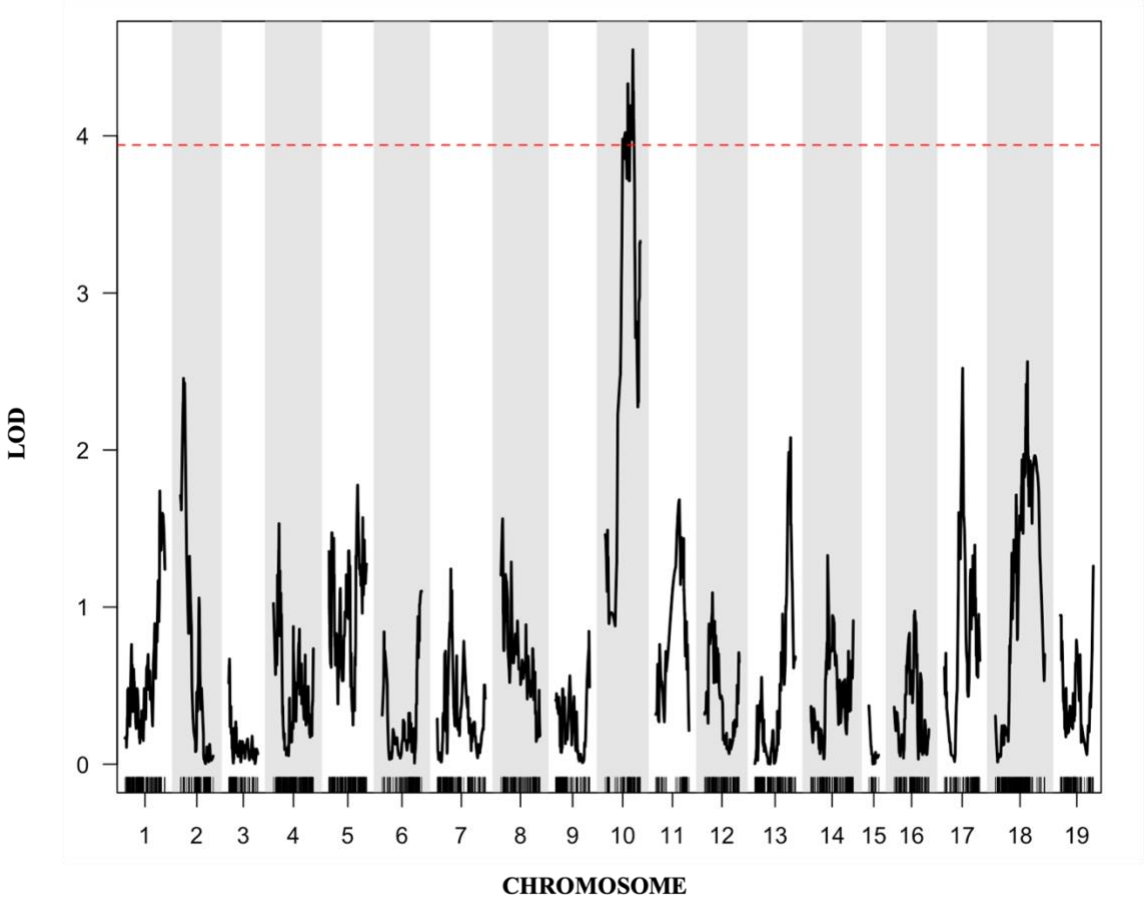

Figure 3-2: QTL showing bud break rate using AUCPC on chromosome 10.
QTL for greenhouse soil-based bud break assay were calculated using SIM in R/qtl, based on 1000 permutations and p-value < 0.05 level of significance.

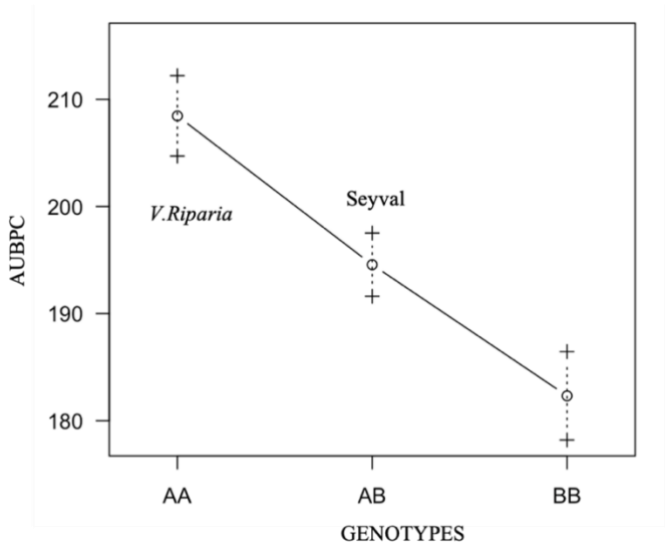

Figure 3-3: AUBPC genotype effect plot for QTL peak marker GBS_10_10801568 on chromosome 10.

Genotype frequency distribution for peak marker GBS_10_10801568 for QTL chromosome 10. Genotype AA represents *V. riparia* female grandparent. Genotype AB represents ‘Seyval’ male grandparent. Values are average AUBPC.

Figure 3-4: Bud break assay in laboratory condition

CHAPTER 4: DEVELOPING AN INTERSPECIFIC POPULATION USING *V.RIPARIA AND V.VINIFERA* ‘ALICANTE BOUSCHET’

4. 1 ABSTRACT

The grapevine is one of the most important economic perennial fruit crops that are extensively grown around the world. The fruit produced is mainly processed into wine but a significant portion of it has been also used for fresh consumption and raisin or processed into juice. The objectives of grapevine breeding can differ according to its use and region growing. However, most breeding programs aim high yield, high fruit quality with improved resistance to multiple diseases and pests, and increased adaptation to hard climate conditions. These desirable traits can be obtained from the genetic resources of *Vitis* genus. Different breeding methods can be used such as conventional breeding methods, mutation, and biotechnological methods to obtain desirable traits. A new population of grapevine with cold hardiness and improved fruit quality and tenturier traits was developed from *V.riparia* (female) and Alicante Bouschet (male). This population will be genotyped and used in marker-assisted selections.

4.2 INTRODUCTION

The grapevine is an economically important fruit crop cultivated, and grapes (*Vitis* spp.) are grown for wine, juice, table grapes, and raisins around the world. While the cultivated species *Vitis vinifera* is the predominantly used species in the industry, other wild grape species contribute importantly to commercial production. Due to its importance, grape breeding and selection have been conducted since its domestication. The grapevine (*Vitis*) is one of the earliest domesticated perennial fruit crops (7000 years ago), around the world (LIANG *et al.* 2019; PATEL *et al.* 2020). In grapevine, morphological traits such as berry size, color, perfect flower, and sugar content appeared as important traits especially in *V.vinifera* during the domestication process. However, with increased climatic challenges and pest and disease problems, breeders need to breed cultivars resistant to biotic and abiotic stress (GRAY *et al.* 2014). The first breeding activities targeting stress tolerance in grapevine were initiated around the beginning of the nineteenth century, predominantly in North America (EIBACH AND TÖPFER 2015). *Vitis vinifera* vines failed due to severe frost damage and the destruction of the grapevines pests or disease. Breeders started to develop hybrids between the European *V.vinifera* cultivars and native American grapes that are abiotic and biotic stress tolerant to produce the best hybrids with desired features of both species. Many breeders like William W. Valk, Nicholas Herbemont, succeeded to develop newly introduced cultivars called American hybrids during the following decades. In the second part of the nineteenth century, breeding for resistance to phylloxera and mildews was initiated in Europe. These cultivars are an important resource for many breeding programs carried out in different grape-growing countries in the world. In the USA, grapevine breeding programs mostly focused on fruit quality or rootstock traits

in the past. However, traits associated with environmental and economical sustainability are increasingly targeted by breeders to maintain successful grape production (GRAY *et al.* 2014). Grape breeders in the USA focus on developing table and raisin, juice, wine, and rootstock cultivars. One of the major goals of breeding programs in the Northern USA is to acquire high-quality, cold hardy, and disease-resistant wine and table grape cultivars with the help of genetic improvement. *Vitis riparia*, *V. labrusca*, *V. aestivalis*, and *V. cinerea* have been used frequently by breeders for this purpose. *V. riparia* has the largest continental distribution of the North American species and it one of the most commonly used species in grape breeding for introgression of freezing tolerance, disease resistance (powdery mildew, downy mildew and, *botrytis*), and phylloxera resistance (LUBY AND FENNELL 2006). The use of *V.riparia* with its locally adaptive traits in breeding has resulted in new cultivars which incorporate the traits of early ripening, high sugar content and maximum freezing tolerance traits (LUBY AND FENNELL 2006). Thus, breeding using *V. riparia* has been successful.

The major cultivated species is *V. vinifera* and it is species of *Vitis*. Currently, there are between 5,000 and 10,000 cultivars of *V. vinifera* grapevine but only a few are commercially significant for wine and table grape production. Alicante Bouchet is a wine grape variety that has been widely used since 1866. It is a grape with red flesh called teinturier and it is one of the few red-fleshed cultivars (ROBINSON 1986; HE *et al.* 2010). Its pulp is fleshy and juicy and has deep dark red color makes it useful for wine production (HE *et al.* 2010; FALGINELLA *et al.* 2012). As grapevine, Alicante Bouschet performs best under warm climates and it is prone to grape diseases such as downy mildew; thus, it is hard to grow in some regions.

The main step in grape breeding is selecting the specific traits of interest in parental material to produce sufficient genetic variation and establish adequate population size. Some common steps have been used to develop a cultivar, including pollen collection, pollen storage, flower emasculation, pollination, and seed management (EIBACH AND TÖPFER 2015). The selection process can take several decades (20-30 years). The selection aims are usually influenced by climatic conditions and biotic factors. Thus, the first part of the selection in the breeding cycle is predominantly concentrated on resistance traits whereas quality traits are often focused on more to the end of the breeding cycle. The limitations in grapevine breeding are mostly owing to crop-specific limitations, access the suitable genetic resources, and lack of genetic information of grapevine (GRAY *et al.* 2014). However, grapevine breeding has been improved and accelerated by the advent of next-generation sequencing and new breeding technologies such as the development of linkage maps with molecular markers.

Grape is one of the most economically important fruit crops in the world, thus grape breeding has been focused on improving the quality of grapes as well as resistance to biotic and abiotic stress. This study aimed to develop a cross between *V.riparia* and Alicante to develop a population with teinturier, cold hardiness, and resistance traits.

4.3 MATERIAL AND METHODS

4.3.1 PLANT MATERIAL, GROWTH, AND MAINTENANCE

V. riparia (VRW, female, white grape) and Alicante Bouchet (heterozygote for tenturier) were selected as parents. Plant materials were grown in 15-L pots and were cycled annually through dormancy in a cold room at 4 °C for at least 150 days. In spring, ecodormant vines were root pruned and repotted in 1:2:2 soil media (soil:peat:perlite by

volume) to maintain uniform growth through the study. The grapevines were grown with a 25/20°C day/night temperature in climate-controlled greenhouses from June to October in Brookings, SD, USA (42°N lat). Grapevines were watered daily and fertilized every two weeks. After bud break, three or four shoots of each plant were selected and trained vertically on bamboo stakes.

4.3.2 PROCEDURES IN AN INTERSPECIFIC CROSS

4.3.2.1 COLLECTION OF POLLEN

Pollen was collected from Alicante Bouschet (male parent) due to the difference in flowering time between *V.riparia* and Alicante Bouschet. Alicante Bouschet is slow to break bud and flower and *V.riparia* breaks bud quickly and flowers very early. Therefore, Alicante Bouschet was forced earlier than *V.riparia*, and pollen was collected for future crosses.

Flower clusters were collected when the bloom was approximately 5% to 20% on the cluster. Clusters were rubbed on the sieve, size 8 above. A cafeteria tray lined with aluminum foil. The clusters were put inside the sieve and rubbed with hands. Flowers are broken off the rachis and pass through the sieve. Flowers were left to dry in about 24 to 30 hours under a lamp (60 W). After that, flowers were chopped in a coffee grinder to release pollen from whole dried flowers. Then, the dried and chopped flowers were put in 50 ml tubes and stored in a -20 °C freezer to use for pollination.

4.3.2.2 POLLINATION AND FRUIT SET

After pollen collection, the *V.riparia* vines were removed from cold storage and grown in the greenhouse. Pollination was conducted by brushing the pollen from the dried and chopped flowers when the majority of the flowers had opened (Figure 4-1). After

pollen was thoroughly applied to a cluster, a translucent paper sleeve was placed over the cluster to prevent fertilization by other pollen sources and labeled.

4.3.2.3 SEED MANAGEMENT

Harvesting of clusters was carried out when physiological ripeness of berries of the berries was reached and seeds were hard and brown. Each cluster from the cross was harvested. Berries were removed from clusters and squished to extract seeds and remove the flesh of the fruit. Seeds were rinsed in water to remove the pulp and transferred to a paper towel to dry. Once they dry, they were counted and put in 50 ml labeled tubes to store at 4°C for breaking of dormancy before sowing (Figure 4-2).

4.4 FUTURE WORK

The stratified seed will be planted in small peat pots, germinated in the greenhouse, and leaves will be collected for genotyping after at least 4 true leaves emerge. After 6 weeks, the wines will be transplanted in the field nursery, grown for one year, and then transplanted to evaluation plots.

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4.6 FIGURES

Figure 4-1: Pollination of flower, applying stored pollen with a brush.

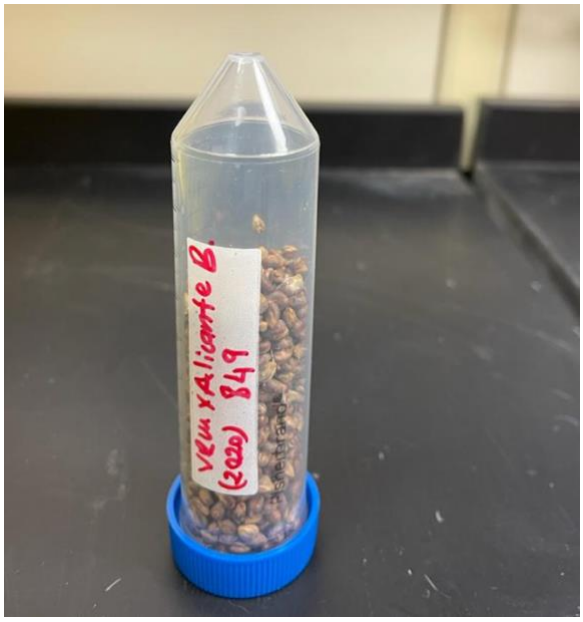

Figure 4-2: Storage of seeds at 4°C.