# EVALUATION OF GENETIC RESISTANCE TO COMMON BACTERIAL BLIGHT IN DRY EDIBLE BEAN 

A Thesis<br>Submitted to the Graduate Faculty of the<br>North Dakota State University<br>of Agriculture and Applied Science

## By

Maniruzzaman

In Partial Fulfillment of the Requirements for the Degree of MASTER OF SCIENCE

Major Department: Plant Pathology

October 2015

Fargo, North Dakota

# North Dakota State University Graduate School 

Title

EVALUATION OF GENETIC RESISTANCE TO COMMON BACTERIAL BLIGHT IN DRY EDIBLE BEAN
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The Supervisory Committee certifies that this disquisition complies with North Dakota State University's regulations and meets the accepted standards for the degree of

## MASTER OF SCIENCE

## SUPERVISORY COMMITTEE:

Dr. Julie Pasche
Chair
Dr. Jack Rasmussen
Dr. Sam Markell
Dr. Juan Osorno

Approved:

October 30, 2015
Date
Dr. Jack Rasmussen
Department Chair


#### Abstract

Common bacterial blight (CBB) is an economically important disease of dry bean worldwide caused by Xanthomonas axonopodis pv. phaseoli, (Xap). The objectives of this research were to determine the frequency of CBB resistance in NDSU breeding materials and to evaluate the effectiveness of two SCAR markers, SAP6 and SU91, linked with major QTL for CBB resistance, across this host population. A total of 593 advanced and preliminary lines were phenotyped in the greenhouse and genotyped using SAP6 and SU91. Phenotyping revealed CBB resistance in 310 lines, with a higher frequency of resistant lines in the pinto, great northern and small red market classes. A total of 188 lines were phenotyped under field condition and only 23 lines were found resistant. The presence of the SU91 marker, and both markers in combination, more effectively identified CBB resistance than did the SAP6 marker alone. Identification of resistant lines should accelerate breeding efforts.


## ACKNOWLEDGEMENTS

I would like to express my heart-felt gratitude and profound appreciation to my major advisor Dr. Julie Pasche whose supervision and suggestions led to completion of this thesis. Special thanks to my thesis committee members Dr. Jack Rasmussen, Dr. Juan M. Osorno and Dr. Samuel Markell for their constructive comments and valuable guidance. I am grateful to Dr. Kristin Simons and Robin Lamppa for their cordial help throughout research and study. I am grateful to all the members of Dry bean and Pulse Lab team. My sincere appreciation to Dr. Kishore Chittem for his assistance in data analysis. In addition, I would like express my deep appreciation to all the faculty members, staffs, and graduate students in the Department of Plant Pathology and my friends of Plant Sciences Department. My sincere gratitude to Dr. Mukhlesur Rahman, faculty of Plant Sciences Department for his support and suggestions. Many thanks to my junior brothers Raihan, Mizan, Arif and Raquib for their inspiration throughout my study. My special thanks to the Northarvest Bean Growers Association and the North Dakota Dry Edible Bean Seed Growers Association for funding this project. I am proud to express my heartfelt appreciation to my mum, dad, my beloved daughter and wife for their love, inspiration, encouragement and endurance throughout my study period.

Finally, it is by the grace of almighty Allah and His abundant love that has made it possible to accomplish this work. The road was too rough but Allah made it smooth and I am proud to Praise His Holy name.

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## LITERATURE REVIEW

## Dry Edible Bean (Phaseolus vulgaris)

Importance. Dry bean is the most important grain legume for direct human consumption and is made up of complex carbohydrates (fiber, resistant starch and oligosaccharides), vegetable protein ( $\sim 22 \%$ ), vitamins and minerals such as calcium, copper, magnesium, manganese, zinc, folate, iron and antioxidants and is very low in fat (Ince and Karaca, 2011; McClean et al., 2004; Miklas et al., 2006). In developing countries, small scale farmers play a major role in dry bean production.

Growth habit. In general, dry beans show two basic growth types, determinate (bush) and indeterminate (vining or trailing) which are further classified as Type I: determinate bush; Type II: indeterminate; upright short vine, with narrow plant profile and 3 to 4 stems; Type III: indeterminate with prostrate vine; and Type IV: indeterminate with strong climbing affinities. Plant development stages are separated into vegetative (V1 to V5) and reproductive (R1 to R9) (Helm et al., 1990).

Production. The total annual world production of dry bean is about 12 million MT. The United States is the sixth-leading producer of dry bean (CGIAR, 2014; USDBC, 2015). Brazil is the largest producer and consumer of dry bean in the world. Latin America leads the world in dry bean production with about 5.5 million MT, where Brazil and Mexico are the main producers. Africa is second in production with about 2.5 million MT, where major leading countries are Uganda, Kenya, Rwanda, Burundi, Tanzania, and Congo. In North America, the United States produces 1.32 million MT, Mexico produces 1.15 million MT and Canada produces 0.245 million MT, (USDA-NASS, 2015, Agriculture and Agri-Food, Canada, 2015, AgroChart, 2015).

Presently, 13 dry bean market classes are commonly grown in the US. The main classes by total production are: pinto, navy, black, dark red kidney, light red kidney, great northern, pink, small red, white kidney and cranberry (USDA-NASS, 2015). Eighteen states produce dry bean on a commercial scale. North Dakota, Michigan, Nebraska, Minnesota, Idaho and California are the top six dry bean producing states in the US. North Dakota leads the US with $32 \%$ of total dry edible bean production, and has produced six market classes for the last five years. Among these, North Dakota produces $54 \%$ of pinto and $40 \%$ of navy beans in the US. Minnesota produces $10 \%$ of the total US dry edible beans and leads the nation in kidney beans (52\%) produced (USDA-NASS, 2014).

Taxonomy. Dry bean (Phaseolus vulgaris L.) is a diploid ( $2 \mathrm{n}=2 \mathrm{x}=22$ ) species of the subtribe Phaseolinae, tribe Phaseoleae, subfamily Papilionoideae, family Fabaceae (Leguminoseae), order Fabales and is predominantly self-pollinating (Ince and Karaca, 2011; Debouck, 1991). Phaseolinae contains five economically important genera; among them Phaseolus is the most widely cultivated genus consisting of about 70 species (Ince and Karaca, 2011). Cultivated species within this genus include P. vulgaris L., P. coccineus L., P. polyanthus Greenman, P. lunatus L., and P. acutifolius A. Gray. (Debouck, 1991). Disease resistance has been identified among $P$. acutifolius A. Gray, P. coccineus L., and P. vulgaris L (Debouck, 1991; Singh and Muñoz, 1999).

Genetic background. Molecular, physiological and morphological research indicates that dry bean evolved from a common ancestral population more than 100,000 years ago and diverged into two genetically distinct geographically isolated gene pools known as MiddleAmerican and Andean (Schmutz et al., 2014). Domestication occurred independently within each of these gene pools about 8,000 years ago. Results of domestication typically included increased
seed and leaf size, changes in growth habit, and photoperiod response. Local adaptations led to various seed coat color and patterns which can now be used to distinguish classes of cultivated adapted beans.

The Middle-American gene pool is categorized in to four races, Durango, Jalisco, Mesoamerica and Guatemala. Race Durango contains pinto and great northern beans (Kelly, 2010). Mesoamerica contains navy and black beans. The market classes, pink, small red, Flor de Mayo and Flor de Junio (Mexico), belong in the race of Jalisco. The Guatemala race contains only climbing beans. The Andean gene pool contains three races, Nueva Granada, Peru and Chile. Light red kidney, dark red kidney, white kidney and bush cranberry beans belong to the race Nueva Granada. The Peru race includes yellow beans. The vine cranberry beans and array of unique beans represent the race Chile in the Andean gene pool. These races show diverse ecological adaptation, geographic range, agronomic traits, allozyme alleles, and random amplified polymorphic DNA markers (Bellucci et al., 2014).

## Development of Dry Beans (Advanced and Preliminary Lines) at NDSU

A modified pedigree method is used to develop dry bean cultivars in the NDSU breeding program. The $\mathrm{F}_{1}$ seeds are produced from crosses in the greenhouse in Fargo, ND. The plants are evaluated in New Zealand in the winter on the basis of phenotypic appearance and vigor. $\mathrm{F}_{2}$ seeds are selected and evaluated in North Dakota the following growing season. The selected best progeny (individual plants) are grown as $\mathrm{F}_{3}$ in rows in Puerto Rico the winter of year two. The best progenies of $\mathrm{F}_{3}$ are again developed as $\mathrm{F}_{4}$ rows back in North Dakota. Based on vigor and appearance 3 to 4 plants are selected from the $\mathrm{F}_{4}$ population and bulked. The F5 population are grown in Puerto Rico in winter. The selected rows are bulked to evaluate important and favorable quantitative traits which are identified as preliminary yield trials (PYT) for yield
testing and overall agronomic performance. Later these preliminary lines are tested across multiple locations over multiple years in North Dakota and Minnesota. The most promising lines goes into advanced yield trials (AYT). In the greenhouse disease screening is conducted for these breeding lines of both PYT and AYT. Canning quality is also evaluated for the advance lines. The best lines evaluated through this way progress into variety trials be considered as commercial cultivars. The total breeding way of methodology requires approximately 8 to 9 years to complete, which begins with crossing and finishes as cultivar release.

## Common Bacterial Blight (CBB)

Common bacterial blight (CBB) is one of the most production limiting factors of dry beans in tropical and subtropical production regions worldwide (Duncan et al., 2011; Tar'an et al., 2001; Viteri and Singh, 2014a). CBB was first reported by Beach in 1892 in New York, USA (Fourie, 2002). It is widespread in nature and attacks a wide range of broadleaf and vegetable crops. Dry bean is the principal host of this pathogen (Harveson and Schwartz, 2007). Other hosts include scarlet runner bean ( $P$. coccineus), tepary bean ( $P$. acutifolius), soybean (Glycine max), lablab bean (Dolichos lablab), common lupine (Lupinus polyphyllus), Georgia velvet bean (Stizolobium deeringianum), fuzzy bean (Strophostyles helvula), moth bean (V. aconitifolia), azuki bean ( $V$. angularis), mung bean ( $V$. radiate), and cowpea ( $V$. unguiculata).

The Causal Organism Xanthomonas axonopodis pv. phaseoli (Xap). The genus
Xanthomonas belongs to the Xanthomonadaceae family and order Xanthomonadales (Benson et al., 2009). The bacterium is gram negative, aerobic, single celled, rod shaped ( 0.4 to $0.7 \times 0.7$ to $1.8 \mu \mathrm{~m}$ ), and moves by a single polar flagellum (Karavina et al., 2011). It contains of about 27 species which cause disease in about 400 host plants (Ryan et al., 2011). Taxonomic classification has not been stable within Xanthomonas since it shows phytopathogenic diversity,
but maintains a homogenous phenotype among species and pathovars (Vauterin et al., 2000). Extensive pathogenic variability of Xap has been found in the Americas and Africa, but pathogenic strains exhibit a high degree of host specificity (Ryan et al., 2011, Viteri et al., 2014a). Bacterial growth occurs at minimum temperatures from $5^{\circ} \mathrm{C}$ to $9^{\circ} \mathrm{C}$ and maximum temperatures from $30^{\circ} \mathrm{C}$ to $39^{\circ} \mathrm{C}$ (Dye and Lelliot, 1974). Under adverse conditions, an extracellular polysaccharide layer acts as a hydrophilic barrier (Lilly et al., 1958). The growth and development of $X a p$ can be influenced by factors like foliar age, host physiology, weather and other microflora. Xap is unable to reduce nitrates and produces characteristic yellow colonies on nutrient (NBA), bacterial blight differential (BBD) and yeast dextrose carbonate (YDC) media. It is a weak producer of acids in media enriched with carbohydrates, catalase positive, and does not prefer to use asparagine for carbon and nitrogen sources.

Disease cycle and symptoms. CBB is a warm-weather disease becoming more severe when temperatures range from $28^{\circ} \mathrm{C}$ to $32^{\circ} \mathrm{C}$. Up to $50 \%$ yield loss may occur in a conducive environment (Harveson and Schwartz, 2007; Viteri and Singh, 2014a). CBB is seed transmitted and contaminated seed is an important source of inoculum for both local and global dissemination of the pathogen (Karavina et al., 2011; Bellucci et al., 2014; O'Boyle and Kelly, 2007). Infected seeds show poor germination and weak vigor, and planting infected seed can accelerate an epidemic. The pathogen can also overwinter on infected plant debris and survive as an epiphyte on dry bean plants or other hosts. When the bacterial population density reaches a threshold of $\sim 10^{5}$ to $10^{6}$ colony forming units per gram of leaf tissue, the infection process is initiated (Duncan et al., 2012). Xap typically enters leaves through stomata, hydathodes or wounds. It grows into the intercellular spaces, causing gradual disintegration of the middle lamella. The bacterium can enter the stem through the stomata of hypocotyls and epicotyls and
wounds and penetrate through vascular elements. The bacteria may be extruded via the stomata, providing inoculum for secondary infections. Foliar symptoms of CBB begin with small watersoaked spots on the bottom of leaves and leaflets (Karavina et al., 2011). The spots enlarge and coalesce. Infected areas may become brown, dry and brittle and are surrounded by a narrow zone of lemon-yellow border. Lesions usually develop on the leaf margin and in the interveinal areas of leaf, giving the plant a burnt appearance. In severe infections, defoliation or stem girdling occurs and dead leaves may remain attached to the stem. Plants grown from infected seed may develop stem girdling or rot. Wilting may occur when a high population of bacterial cells plug the vessels of the host cell wall (Harveson and Schwartz, 2007; Karavina, et al., 2011; Saettler, 1989).

Xap may enter into pod sutures from the pedicel connected with the vascular system and enter into the funiculus through the raphe of the seed coat, possibly causing hilum discoloration. Pod symptoms generally appear as circular, sunken, dark-brown lesions, that later turn dark redbrown (Karavina et al., 2011; Harveson and Schwartz, 2007). The size and shape of lesions can vary with pod age. Under conditions of high temperature $\left(28^{\circ} \mathrm{C}\right.$ to $\left.32^{\circ} \mathrm{C}\right)$ and relative humidity ( $60 \%$ or greater), yellow masses of bacteria ooze from pod lesions.

The bacterium can overwinter under or on the seed coat (Karavina et al., 2011). During germination, it may remain on the seed coat or enter the cotyledon. Generally, white or lightcolored seeds produce butter-yellow or brown spots on the seed coat which may be are limited to the hilum area (Karavina et al., 2011). Seed may be rotted, shriveled or wrinkled and discolored when infection occurs in early pod development, which negatively affects commercial quality and value, emergence, and seedling vigor (Harveson and Schwartz, 2007; Mutlu et al., 2005; Vandemark et al., 2009). These symptoms are difficult to identify in dark-colored seeds
(Karavina et al., 2011; Harveson and Schwartz, 2007). Xap can be disseminated from infected to healthy plants, within or to neighboring fields, by wind, wind-driven rain, irrigation water, hail storms, people, or machinery (Duncan et al., 2012; Harveson and Schwartz, 2007).

Disease management. The survival and infection process of Xap is complex (Duncan et al., 2011, 2012) and Xap can survive up to ten years in bean seed (O'Boyle and Kelly, 2007). The management of CBB is difficult due to its extended viability on/or in seed and epiphytic nature in the field that limits the effectiveness of crop rotation (Duncan et al., 2011, 2012; Vandemark et al., 2008). Copper based bactericides are not typically used to control the disease since they are phytotoxic, only effective on bacteria on plant surfaces and most effective when applied prior to infection (Agrios 2005). Antibiotics are not generally recommended due to cost, potential of the development of resistance in the pathogen and public concern. An integrated pest management (IPM) approach can help to limit outbreaks of the disease. An IPM approach begins with planting disease-free seed, crop rotation, soil incorporation of plant debris, not planting next to recently blighted fields, avoiding plant injury, not walking through or moving machines when the canopy is wet, and not re-using irrigation water (Duncan et al., 2012; Harveson 2007). However, these practices may not provide adequate control under high disease pressure. Genetic resistance is the most effective and efficient tool to provide durable management of CBB (Bett and Banniza, 2014; Duncan, et al., 2011; O’Boyle and Kelly, 2007; Osdaghi et al., 2010).

## Phenotypic Screening

Phenotypic selection is necessary in the development of CBB resistant breeding materials to maintain minor effect QTL and to detect the epistatic interaction contributing to superior CBB resistance (Miklas et al., 2006, Fourie, 2002). Using two isolates of Xap (Colorado and Wisconsin) and their two inoculation concentrations, 31 genotypes were evaluated those were
incorporated CBB resistance from common bean, scarlet runner bean and tepary bean (Lema et al., 2007). Large differences were observed in response to Colorado and Wisconsin isolates, densities and evaluation time period between 14 DAI and 21 DAI. Higher densities of aggressive Wisconsin isolate ( $\geq 10^{8} \mathrm{CFU} / \mathrm{mL}$ ) the CBB resistance were not effective particularly at 21 DAI among the three species in the genotypes of incorporated CBB resistance. But resistance incorporated from tepary bean was found as most effective to CBB resistance. CBB evaluation of 21 lines and one cultivar were conducted in greenhouse and field conditions and reported that none of the lines and/or cultivars were immune to CBB (Osdaghi et al., 2010). Cultivar Khomein and Ks21479 and Ks31169 were found as more susceptible but Ks51103, BF13607 and BF13608 lines were found as most resistant and suggested to use the resistant lines and cultivar as a source of resistance to CBB in plant breeding programs. CBB evaluation was conducted in some cultivars and advanced lines of common bean, tepary bean, lima bean and scarlet runner bean in field using aspersion, surgical blades, and/or multiple needles and reported the $P$. acutifolius accessions, G40029 and G40156 exhibited the highest level of resistant (1.2-2.0) (Singh and Muñoz, 1999). The $P$. lunatus, $P$. coccineus and $P$. vulgaris scored (4.2-6.2), (4.8-5.5) and (4.56.5), respectively and lines with pyramided resistance exhibited higher level of CBB resistance. In the greenhouse and field, CBB resistance was assessed in an inter-gene pool double cross population, Wilkinson 2 X DRK 2 and DRK 1 X VAX 3, under high disease pressure 12 resistant breeding lines were found through phenotypic selection (DDS) whereas 6 lines were obtained through MAS selection (Duncan et al., 2012). The mean disease severity in phenotypic selection (DDS) was 3.3 and in MAS it was 4.2. In cost comparison phenotypic selection was cheaper (US\$ 1.55) than MAS (\$2.03) per plant. Field experiment were conducted in 2003 and 2004 using two resistant cultivars and four susceptible cultivars following non-inoculated and
inoculated of leaf with Xap (Gillard et al., 2009). It was reported that the CBB incidence was very low in the non-inoculated than the inoculated experiment. The resistant cultivars showed low disease severity than the susceptible cultivars. Phenotypic and genotypic screening of some breeding lines developed from donor VAX and RMX lines using Xf260 and Xf410 isolates in greenhouse resulted resistant progeny within population but population means displayed intermediate level of resistance which indicated the quantitative nature of CBB inheritance to Xap (Kachulu et al., 2011). In genotypic analysis revealed SU91 was linked with CBB resistance but SAP6 was not associated with CBB resistance loci. A large number of pathogenic variability and pathogenicity of Xap has been reported in the USA and Africa ((Ryan et al., 2011, Viteri et al., 2014a)). But the pathogenicity does not loss if it cultured either liquid or solid media (Cruz Izquierdo et al., 2001). Using three isolates of Xap the pathogenicity was evaluated in both solid and liquid media of YDC (yeast-dextrose-calcium carbonate-agar). The susceptible cultivars Flor de Mayo Criollo and Negro P20 was inoculated with Xap from both solid and liquid media in same concentration ( $10^{9} \mathrm{CFU} / \mathrm{mL}$ ) and reported Xap can be cultured in liquid media which show the same pathogenicity as solid media.

## Host Genetic Resistance

Three Phaseolus gene pools have been used to introgress genes of interest into adapted dry bean breeding materials. Wild progenitors of $P$. vulgaris (common bean) belong to the primary gene pool; of interest in the secondary gene pool is $P$. coccineous (scarlet runner bean); and of interest in the tertiary gene pool is $P$. acutifolius (tepary bean) (Singh and Schwartz, 2010; Singh and Muñoz, 1999). Only limited low to moderate levels of resistance have been found in the primary and secondary gene pools; however, incorporating increased resistance from tertiary gene pool can be difficult. Successful hybridization between primary and tertiary gene pools
usually requires embryo rescue (Singh and Schwartz 2010; Parker and Michaels 1986). Embryo rescue is not typically necessary for hybridization between the primary and secondary gene pools but the $\mathrm{F}_{1}$ progeny may occasionally exhibit lethality, dwarfism, sterility, and/or an increase in outcrossing (Singh and Schwartz 2010). Resistance typically has been incorporated into $P$. vulgaris through interspecific hybridization with $P$. acutifolius and $P$. coccineous (Durham et al., 2013; Kelly et al., 2003; Singh and Schwartz, 2010).

Presently, available dry bean cultivars, regardless of the market classes, contain little CBB resistance. Low to moderate levels of CBB resistance are observed in Montana No. 5 and $P$. coccineous, whereas $P$. acutifolius has demonstrated the highest levels of resistance (ArnaudSantana et al., 1993; Coyne and Schuster, 1983; Mohon, 1982, Miklas et al., 2003; Singh and Schwartz, 2010). The first hybridization between $P$. vulgaris and P. acutifolius resulted in more than 10,000 advanced-generation progenies following embryo rescue and congruent backcrosses of the interspecific F1 hybrids (Singh and Muñoz, 1999). Congruent backcrossing was used to overcome hybridizations barriers such as genotype incompatibility, early embryo abortion, hybrid sterility and a lower rate of hybridization (Fourie, 2002). The development of the OAC 88-1 (Scott and Michaels, 1992), XAN 159, XAN 160, and XAN 161 (Beebe and PastorCorrales, 1991; McElroy, 1985) breeding lines with higher levels of CBB resistance were generated by using $P$. vulgaris $\times P$. acutifolius recurrent backcross populations. Additional genotypes of $P$. acutifolius were used to develop additional CBB resistant breeding lines, including VAX 1, VAX 2, VAX 3, VAX 4, VAX 5 and VAX 6 (Singh and Muñoz, 1999). The resistance level was higher in VAX 3, VAX 4 and VAX 6 compared to the original P. acutifolius accessions. These lines also displayed improved environmental adaptation, plant type and seed color. The breeding lines HR45 and HR67 were also derived from P. acutifolius through
interspecific crosses. These lines also displayed higher levels of CBB resistance than their parents. These lines are highly resistant to CBB and as well as other diseases like white mold (Park et al., 2007). The white bean cultivar Apex has been developed from HR 67. This high yielding cultivar has exhibited moderate resistance to CBB. Therefore, higher levels of CBB resistance are needed to incorporate into popular dry bean cultivars from all Phaseolus species of the primary, secondary, and tertiary gene pools (Singh and Schwartz, 2010).

## Molecular Markers and QTL

CBB resistance is quantitatively inherited by major-effect quantitative trait loci (QTL) and minor-effect QTL with low to medium heritability of resistance (Aggour et al., 1989; Arnaud-Santana et al., 1994; Silva et al., 1989; Valladares-Sánchez et al., 1979, 1983; Webster et al., 1980; Taran et al., 2001; Tryphone et al., 2013; reviewed in Singh and Schwartz, 2010). Depending on the population used, one to several loci contribute CBB resistance in the leaf and/or the pod. A single genotype can exhibit resistance in leaves but pods may display susceptible reaction or vice versa. Plants may also exhibit and bean plant may provide resistance to some strains but susceptibility to others (Singh and Schwartz, 2010). Breeding for genetic resistance to CBB is challenging not only because the most effective resistance comes from the secondary and tertiary gene pools, but because more than 22 QTL have been identified. These QTL are spread across all 11 linkage groups of dry bean. The effects of these QTL are highly variable depending on disease pressure, environmental conditions, genetic background, maturity of the plant and infection targeted plant organ, such as seed, leaf, and pod (Durham et al., 2013; Kelly et al., 2003; Miklas et al., 2006).

Molecular markers, namely sequence characterized amplified region (SCAR) markers, are widely used to detect QTL conditioning resistance to CBB. Many of these SCARs are
developed from random amplified polymorphic DNA (RAPD) markers and use longer primers during polymerase chain reactions to avoid problems associated with RAPD markers (Paran and Michelmore, 1993), such as poor reproducibility due to extreme sensitivity to reaction conditions. SCAR markers are preferable over RAPD markers because SCAR markers are more robust, more reliable, can easily detect a single locus and may be codominant (Collard et al., 2005; Paran and Michelmore, 1993). SCAR markers are therefore more easily used to improve resistance selection due to multiplex polymerase chain reaction (PCR) and relative ease of scoring for complex inherited CBB resistance (Mutlu et al., 2005).

Two SCAR markers, widely used in marker assisted selection (MAS) and in genetic studies for CBB resistance, are SAP6 and SU91, which are linked with major QTL located on linkage groups, Pv10, and Pv08, respectively (Mutlu et al., 2005; O'Boyle and Kelly, 2007; Vandemark et al., 2008). Unfortunately, these markers are dominant and it is difficult to differentiate plants with only one copy of gene (heterozygous) from plants with two copies of gene (homozygous) (Vandemark et al., 2008). To date, no codominant markers have yet been found which is tightly linked to either SU91 or SAP6.

The SAP6 marker was originally linked to CBB resistance in a population developed between Dorado/Xan176, where Xan176 was a product of a hybridization between landrace Montana No. 5 and tepary bean Honma, 1956). Resistance was thought to originate from the tepary bean (Miklas et al., 2003). Later studies with other descendants from Montana No. 5 including GN Nebraska \#1 and GN Nebraska \#1 Sel. 27, demonstrated that the CBB resistance was derived from the landrace Montana No. 5. In a Montana No. 5 x Othello population, SAP6 accounted for $35 \%$ phenotypic variation for CBB resistance (Miklas et al., 2003). Now this resistance source is widely used to increase the level of resistance in other cultivated varieties.

In the population between Belneb RR-1 breeding line, PR0313-58, and 'Rosada Nativa', SAP6 explained $55 \%$ of the phenotypic variation (Zapata et al., 2010).

The SU91 marker was first linked to CBB resistance in a Dor476 x SEL 1309 population (Pedraza et al., 1997). SEL 1309 was derived from P. acutifolius via Xan 159. The original RAPD marker was linked to a resistance QTL which explained $17.4 \%$ of the phenotypic variation. It was converted to a SCAR marker which then explained $23.7 \%$ of the phenotypic variation. The SU91 SCAR marker has been widely used in MAS for introgression of higher levels of CBB resistance into dry bean (Viteri et al., 2014a, 2014b; O’Boyle and Kelly, 2007). In $\mathrm{F}_{2}$ populations from a cross between XAN 159 X Chase, the presence of SU91 explained $14 \%$ of the phenotypic variation (Vandemark et al., 2008). $\mathrm{A}_{\mathrm{BC}}^{2}$ F $\mathrm{F}_{1}$ population from that same cross exhibited $17 \%$ phenotypic variation. In the cross between DOR476 X SEL1309, 24\% phenotypic variation in CBB leaf reaction was correlated to the presence of SU91 markers and 25\% phenotypic variation explained in the XAN159 X Teebus population (Pedraza et al., 1997). The position of SU91 and SAP6 on different linkage groups allows them to be used to reliably incorporate independent QTL conditioning resistance to CBB into susceptible dry bean cultivars (Vandemark et al., 2008).

Recently, a new CBB resistance QTL, Xa11.4, was found on linkage group Pv11.4, in the interspecific breeding line VAX1. It was derived from tepary bean G40001. In the cross between Othello $\times$ VAX 1, this new QTL accounted $45 \%$ and $51 \%$ phenotypic variance in primary and trifoliate leaves, respectively against the Xap isolate, Xcp25 (Viteri et al., 2014b). But in the cross between Othello $\times$ VAX 3, $26 \%$ and $37 \%$ phenotypic variation was observed in primary and trifoliate leaves respectively, against the same isolate. Xa11.4 accounted $23 \%$ and $18 \%$ phenotypic variance in primary and trifoliate leaves, when inoculated with the Xap isolate

ARX8AC respectively in the cross between Othello $\times$ VAX 1, while $13 \%$ and $22 \%$ phenotypic variance was observed in primary and trifoliate leaves, respectively in the cross between Othello $\times$ VAX 3. This QTL has shown higher level of CBB resistance than the QTL linked to SU91 and SAP6 (Viteri et al., 2014b). Multiplexing these markers in a single PCR reaction to use in MAS for combined CBB resistance from multiple QTL, could be a promising tool to confer higher levels of resistance to CBB (Miklas, 2000; Miklas et al., 2003; Mutlu et al., 2005).

To date, more than 22 QTL have been identified, nevertheless, high levels of CBB resistance have not been widely incorporated into dry bean (Duncan et al., 2011). This is due to linkages between resistance QTL and undesirable traits, such as low yield and defective seed size or color, and hybridization barriers such as genotype incompatibility, early embryo abortion, hybrid sterility and lower rate of hybridization (Duncan et al., 2011; Singh and Muñoz, 1999; Yu et al., 2000). Therefore, knowledge of the interaction of QTL and a clear understanding of the mode of inheritance and resistance gene expression in the developed materials is a prerequisite for a CBB resistant breeding program (Durham et al., 2013). Proper caution must be used when choosing and using markers for MAS based on the quality and number of available markers, resistance donor germplasm, and market class to be improved (Singh and Schwartz, 2010). Much larger populations and a large number of markers defining major and minor effect QTL for CBB resistance may need to be used for MAS. However, using the tightly linked CBB resistance molecular markers with direct disease screening and using the most aggressive pathogen, can accelerate the breeding for CBB resistance (Lema et al., 2007).

## Evaluation of Breeding Materials Using Phenotypic Selection

Phenotypic selection is necessary in the development of CBB resistant breeding materials, to maintain minor effect QTL and to detect the epistatic interaction contributing to
superior CBB resistance (Miklas et al., 2006, Fourie, 2002). Many factors are involved to enhance the disease expression to aid in phenotypic screening such as inoculation method, age and concentration of inoculum and experiment. There are several inoculation methods have been developed such as aspersion or spray (inoculation sprayed under pressure on leaves) and wounding of leaves using scissors, razor blades, needles, surgical blades and water soaking (Aggour et al., 1989; Fourie, 2002). Currently, the sprayer method is widely used for disease evaluation in dry bean (O'Boyle and Kelly, 2007, Singh and Muñoz, 1999) because of its ease of use. The concentration and age of inoculum is an important factor during inoculation of plants with Xap. The optimum concentration of inoculum is $10^{6}$ to $10^{10} \mathrm{CFU} / \mathrm{mL}$ (Cruz Izquierdo et al., 2001). A suspension of bacterial inoculum with too low a concentration may result in a susceptible individual may show moderately resistance, conversely, inoculum that is too concentrated may result in a moderately resistant individual being deemed susceptible (Gilbertson et al., 1988; Fourie, 2002). The inoculum should be prepared from fresh cultures less than 48 hours and used within (1-2) hours of preparation.

Plant characteristics including growth habit, phenological stage and handling after inoculation (Coyne and Schuster, 1983) can affect phenotyping results. In dry bean, trifoliate leaves typically display higher disease severity than primary leaves (Viteri et. al., 2014a). In greenhouse experiments, CBB is commonly more severe than in field experiments (Duncan et al., 2012; Miklas et al., 1996; Vandemark et al., 2009). The greenhouse environment typically is more favorable for disease development and inoculation is done by directly wounding the leaf tissue, which enhances the pathogen entrance into plant tissue. Under field conditions, disease severity will increase with early infection and favorable environmental conditions (Lema et al., 2007). Standardization of inoculation methods is challenging due to a wide range of
environments where evaluations are conducted and variability in laboratory and field facilities
(Gilbertson et al., 1991). Evaluation of phenotype is complicated by various interactions among these factors, in addition to the loss of pathogenicity or differences in bacteria handling among staff and laboratories (Fourie, 2002). This makes MAS valuable in selecting lines for CBB resistance.

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# CHAPTER 1: EVALUATION OF DRY BEAN BREEDING MATERIAL FOR RESISTANCE TO COMMON BACTERIAL BLIGHT 

## Introduction

Dry bean is one of the main legume crops in Latin America, North America, Africa and Asia. It is a major source of protein, vitamins, minerals, and fiber. Most poor people in Africa and Latin America consumed it as a primary staple food (Bitocchi et al., 2012; Miklas et al., 2006a; Tryphone et al., 2013; Yu et al., 2012). Dry bean belongs to the genus Phaseolus which contains about 70 species. Phaseolus contains five economically important species, these are common bean ( $P$. vulgaris), year-long bean ( $P$. polyanthus), scarlet runner bean ( $P$. coccineous), tepary bean ( $P$. acutifolius) and the lima bean ( $P$. lunatus). Among these five domesticated species, $P$. vulgaris contributes more than $90 \%$ of cultivated crop worldwide and the most consumed grain legume in the world (Debouck, 1991; Ince and Karaca, 2011). North Dakota leads the US in production of dry beans. It has produced an average of $32 \%$ of the total US dry beans in the last five years (NASS, USDA, 2014).

Common bacterial blight (CBB) caused by Xanthomonas axonopodis pv. phaseoli (Smith) Vauterin et al. (Xap) is a major production limiting factor of dry beans. Xap is seed transmitted and contaminated seed is an important source of inoculum for both local and global dissemination of the pathogen (Mutlu et al., 2005; Singh and Schwartz, 2010; Tar'an et al., 2001; Mutlu et al., 2008). Yield loss of up to $50 \%$ in tropical and subtropical regions of the world has been reported in conducive environments (Viteri and Singh, 2014a). The amount of yield loss is influenced by factors including disease severity, plant age at infection period, degree of susceptibility of cultivars and environmental conditions (Osdaghi et al., 2010; Saettler, 1989, Singh and Muñoz, 1999). Planting pathogen-free seed, the application of a foliar bactericide,
crop rotation with a non-host species and deep ploughing can limit CBB epidemics, but these practices often do not provide economically adequate disease control in many instances (O'Boyle and Kelly, 2007). The most promising and effective method for controlling CBB is to grow resistant cultivars (Osdaghi et al., 2010; Saettler, 1989, Singh and Schwartz, 2010).

Substantial efforts have been put forth to identify CBB resistant dry bean genotypes using molecular markers. Molecular markers provide benefits over selection based on the phenotypic disease screening, which may be affected by several environmental factors (Kelly et al., 2003; Tryphone et al., 2012, Tryphone et al., 2013; Yu et al., 2012;). Molecular markers can be more effective over conventional methods in selecting for quantitative and low heritability traits (Miklas et al., 2006a). MAS has a wide range of utilization in plant breeding, which is classified into four types (Xu and Crouch, 2008). First, MAS is beneficial for the traits that are challenging to maintaining through phenotypic screening. This may be due to either cost or complex inheritance. Second, MAS is better than phenotypic screening when conventional selection is highly dependent on environments and the developmental stage of the host. Third, MAS is more effective in selecting recessive alleles during backcrossing. Finally, MAS is more feasible in pyramiding multiple QTL for a single trait with complex inheritance (Xu and Crouch, 2008). Furthermore, the advantage of MAS is it reduce time, money and effort, increased consistency and efficiency, and enhanced biosafety (Jena and Mackill, 2008).

CBB resistance has been reported in the primary, secondary and tertiary gene pools of dry bean (Singh and Muñoz 1999; Urrea et al., 1999; Welsh and Grafton, 2001) but little or no CBB resistance is present in most commercial dry bean cultivars. In addition, CBB resistance QTL are derived from a broad spectrum of breeding lines of common, tepary and scarlet runner
beans (Miklas et al., 2006a). Tepary bean is reported as a major source of CBB resistance QTL and is considered to be in the tertiary gene pool to common bean (Yu et al., 2012).

SAP6 is a dominant sequence characterized amplified region (SCAR) marker on linkage group, Pv 10 , that is linked to a CBB resistance QTL and identified in the great northern bean line GN Nebraska No. 1 sel. 27 (Kelly et al., 2003; Miklas et al., 2006a). GN Nebraska No. 1 sel. 27 was developed from an interspecific cross between Montana No. 5 and tepary no. 4 (Kelly et al., 2003; Miklas et al., 2006a) where resistance was assumed to be derived from the tepary parent. Later research found this QTL was not present in the tepary parent and was therefore derived from Montana No. 5 (Jung et al., 2014; Kelly et al., 2003; Miklas et al., 2006a; Miklas et al., 2003). The SAP6 linked QTL explained $35 \%$ of the phenotypic variation to CBB reaction in the Montana No. 5 population and it is now widely used for increased level of resistance in other cultivated varieties (Miklas et al., 2003).

SU91 is also a SCAR marker located on linkage group Pv08. Pedraza et al., (1997), reported $24 \%$ phenotypic variation in CBB leaf reaction correlated to the presence of SU91 markers in the DOR476 $\times$ SEL1309 and $25 \%$ phenotypic variation in the XAN159 $\times$ Teebus population (Yu et al., 2012). In XAN $159 \times$ Chase $\mathrm{F}_{2}$ populations, the presence of the SU91 marker explained $14 \%$ phenotypic variation and $17 \%$ phenotypic variation was explained in the $\mathrm{BC}_{2} \mathrm{~F}_{1}$ population (Vandemark et al., 2008). Both phenotypic and genotypic data should be applied in selecting resistance genotypes due to the complexity of CBB resistance inheritance and the environmental influences (Miklas et al., 2003; Viteri et al., 2014a, 2014b).

The objectives of this research are to i) determine the frequency of CBB resistance among advanced and preliminary dry bean lines from the NDSU breeding materials important to North Dakota and Minnesota producers through genotyping and phenotyping, ii) evaluate the
usefulness of the SAP6 and SU91 markers across varying market classes and genetic backgrounds.

## Materials and Methods

Plant materials. A total of 593 genotypes which includes 85 advanced and 425 preliminary lines in the Middle-American market class and 83 preliminary lines in the Andean market class were evaluated in the greenhouse for resistance to CBB (Table 1.1). These are NDSU breeding materials. NDSU is developing dry bean cultivars of several market classes on aspects such as yield and disease resistance to meet the needs of North Dakota and Minnesota producers.

Table 1.1. The number of genotypes of across market classes among the Middle-American and Andean genetic background evaluated for resistance to common bacterial blight under greenhouse conditions.

| Middle-American |  | Andean |  |  |
| :--- | :--- | :--- | :--- | :--- |
| Market Class | Advanced lines | Preliminary lines | Market Class | Preliminary lines |
| Pinto | 11 | 171 | Dark Red Kidney | 37 |
| Navy | 14 | 40 | Light Red Kidney | 31 |
| Black | 30 | 70 | White Kidney | 15 |
| Great | 19 | 65 | - | - |
| Northern |  |  | - | - |
| Small red | 6 | 60 | - | - |
| Pink | 5 | 19 |  | 83 |
| Total | 85 | 425 |  |  |

The pathogen Xapf91-5. Bacterial isolate Xap f91-5 was used for phenotyping in the greenhouse trials. In 1991 few isolates were collected by the dry bean and pulse pathology lab based on field CBB symptoms across North Dakota dry bean fields. The collected isolates were tested and among them the isolate Xap f91-5 was highly virulent.

Phenotypic evaluation in greenhouse condition. Experimental design: The experiment was set in randomize complete block design with three replicates. One seed in each replication and 593 advanced and preliminary lines was planted in a block and the experiment was performed three times.

Development of plant population: Seeds of each line were planted as described above and plants were fertilized with water soluble Peat-lite (20-20-20) one teaspoon per liter once a week. The beneficial nematode Nemasys (BASF) was applied once a week at $150 \mathrm{ml} / 1650 \mathrm{ft}^{2}$ for thrip control. The greenhouse light was adjusted to 600 watt (High pressure sodium) for 16 hours and temperature ranged from $28^{\circ} \mathrm{C}$ to $32^{\circ} \mathrm{C}$ for 24 hours.

Inoculum preparation and inoculations: The bacterium was grown for 2 to 3 day at $28^{\circ} \mathrm{C}$ on nutrient agar (Nutrient brooth $13 \mathrm{~g} / \mathrm{L}$ and Bacto agar $15 \mathrm{~g} / \mathrm{L}$, dissolved in sterile water) (Aggour et al., 1989; Miklas et al., 1996; Osdhagi et al., 2009) and diluted in 0.0125M potassium phosphate buffer ( pH 7.1 ) (Miklas et al., 1996, Mutlu et al., 2008) to $1 \times 10^{7}$ to $1 \times 10^{8} \mathrm{CFU} / \mathrm{mL}$ (Duncan et al., 2012; Fourie and Herselman, 2011; Miklas et al., 2011) using a Jenway 7300 spectrophotometer. The abaxial surface of the first trifoliate leaves $\left(20-30 \mathrm{~cm}^{2}\right)$ were inoculated using an air brush sprayer 19 to 21 days after planting (V1 stage) (O’Boyle et al., 2007; Singh and Munoz, 1999; Tryphone et al., 2012). The susceptible cultivar, Othello (does not contain SAP6/SU91 marker) was used as susceptible check (Miklas et al., 2003), and the resistant breeding line, XAN 159, which contains SU91, were used as resistant check (Singh and Muñoz; Vandemark et al., 2008). Both of these checks were inoculated for every 84-120 lines evaluated for CBB reaction. The inoculated plants were transferred to misting chambers and maintained at $>90 \%$ relative humidity for 48 h and returned to the greenhouse until scoring for reaction to CBB. Disease reactions were evaluated 14 and 21 days post-inoculation (dpi) using 1 to 9 scale
(Aggour et al., 1989) where 1 is no visible reaction and 9 is highly susceptible (Table 1.2; Fig.

## 1.1).

Table 1.2. Disease reaction rating scale for common bacterial blight in dry bean used to identify the resistant and susceptible genotypes of advanced and preliminary lines in Andean and Middle-American genetic background during the greenhouse study.
\% of inoculated leaf area with necrotic lesion and/or chlorosis (leaf area Rating scale ${ }^{y}$ affected) ${ }^{\mathrm{x}}$

| No necrotic lesion and/or chlorosis | 1 |
| :--- | :--- |
| $1-12.5$ | 2 |
| $13-25.5$ | 3 |
| $26-38.5$ | 4 |
| $39-51.5$ | 5 |
| $52-64.5$ | 6 |
| $65-77.5$ | 7 |
| $78-90.5$ | 8 |
| 91 to 100 | 9 |
| "x" represents \% of leaf area necrosis/chlorosis, "y" represents disease rating 1-3 = resistant, >3- |  |
| 6 = intermediate resistant, $>6-9=$ susceptible (Aggour et al., 1989). |  |



Figure 1.1. Disease rating scale for common bacterial blight in dry beans used to identify the resistant and susceptible genotypes of advanced and preliminary lines in Andean and MiddleAmerican genetic background during the greenhouse study (Aggour et al., 1989).

Phenotypic evaluation in field condition. The field evaluation was conducted in Prosper in 2014 using 188 advanced (54) and preliminary (134) lines. The field trial was planted in midMay 2014 and it was infected with Xap naturally. CBB disease severity was scored in the beginning of August 2014 using the same disease severity scale (1-9) (Aggour et al., 1989) used in greenhouse evaluation.

Genotypic evaluations. DNA Extraction: A total of 593 genotypes which includes 85 advanced and 425 preliminary lines in the Middle-American market class and 83 preliminary lines in the Andean market class were planted in the greenhouse for genotyping (Table 1.1). Seeds were surface sterilized with a $1.2 \%$ sodium hypochlorite solution, placed on solid media
containing 2\% agar in Petri plates, and incubated at room temperature to allow germination. After 4 to 5 days, germinated three seeds of each line were planted in $4 \times 4 \times 4.5$ pots containing Peat-based growing mix-LC8 (Canadian Sphagnum peat moss 70-80\%, Perlite 20-25\%, Vermiculite $5-10 \%$ ) in the greenhouse. The plants were fertilized with water soluble Peat-lite (20-20-20) one teaspoon per liter once a week. After two weeks, a leaf was collected from all plants, freeze dried and grounded to a powder. DNA was extracted using a modified cetyltrimethyl ammonium bromide (CTAB) method with some modifications (Doyle and Doyle, 1990). Carlson buffer was used for DNA extraction and chloroform: isoamyl alcohol was not used for cleaning the DNA sample (Carlson et al., 1991). The extracted DNA was quantified in $0.8 \%$ agarose gel and adjusted to approximately $100-150 \mathrm{ng} / \mu \mathrm{l}$ prior to conducting PCR.

Molecular marker evaluations: PCR was conducted using the two dominant SCAR markers, SAP6 and SU91 (Miklas et al., 2000; Yu et al., 2000). Each reaction contained 100-150 ng genomic DNA, 0.188 mM each dNTPs (Promega), $0.25 \mu \mathrm{M}$ forward and reverse primer, 1 U GoTaq DNA polymerase (Promega), and $1 \times$ GoTaq Buffer (Promega) in a total volume of $20 \mu \mathrm{l}$. PCR was performed using the following amplification profile for SAP6; 10 s at $94^{\circ} \mathrm{C}, 40 \mathrm{~s}$ at $55^{\circ} \mathrm{C}, 120 \mathrm{~s}$ at $72^{\circ} \mathrm{C}$ for 34 cycles followed by one cycle for 5 min at $72^{\circ} \mathrm{C}$. The same amplification profile was used for SU91 except an annealing temperature of $58^{\circ} \mathrm{C}$ was used. In this study, the black bean variety T-39 was used as positive amplification control for SAP6 since it possesses SAP6 marker and small red line VAX3 (Duncan et al., 2012) for SU91. Lariat was included as a negative amplification control for both markers. The amplified PCR products were separated on a $2 \%$ agarose gel. Amplification was recorded as positive if a $\sim 820 \mathrm{bp}$ product was observed in the SAP6 reaction (Fig 1.2) and a $\sim 700 \mathrm{bp}$ product in the SU91 reaction (Fig 1.3).


Figure 1.2. Gel picture of SAP6 marker ( 820 bp amplicon) " 1 " represents positive control and " $2-16$ " represents dry bean line with and without SAP6 marker ( 820 bp ) and "M" represents marker.


Figure 1.3. Gel picture of SU91 marker ( 700 bp amplicon) " 1 " represents positive control and "2-16" represents dry bean line with or without SU91 marker (700 bp) and "M" represents marker.

Statistical analysis. The experiments were conducted in a randomized complete block design with three replicates. The mean of the reaction of all plants in each replicate were calculated. To determine the significant difference between dry bean advanced and preliminary lines and among the two genetic backgrounds (Middle-American and Andean) and market classes (pinto, navy, black, small red, great northern, pink, dark red kidney, light red kidney and white kidney) data were analyzed using GLM procedure in SAS (Version 9.3, SAS Institute). Mean separations were conducted using Tukey-Kramer least square means separation test. Pearson correlation coefficient values were calculated to compare the field and greenhouse CBB disease severity with the CORR procedure in SAS.

## Results

Phenotypic analysis of CBB resistance in greenhouse conditions. The data collected at 14 days post inoculation (dpi) demonstrated little difference between the CBB resistant and susceptible check and was not analyzed further. The data collected at 21 dpi demonstrated differences between the resistant and susceptible check and was included in the analysis. Among
all 593 lines evaluated for reaction to Xap under greenhouse conditions, 310 lines (52\%) were resistant ( 1 to $3=1-25.5 \%$ necrosis/chlorosis), 255 lines ( $43 \%$ ) were intermediate ( $<3-6=25.6-$ $64.5 \%$ necrosis/chlorosis) and only 28 lines (5\%) were susceptible ( $>6-9=64.6-100 \%$ necrosis/chlorosis) (Table 1.3; Table 1.4; Table 1.5). In the advanced lines of Middle-American genetic background, a larger percentage of great northern (84\%), black (77\%), navy (71\%), small red ( $67 \%$ ) and pink ( $60 \%$ ) lines were resistant compared to susceptible (Table 1.3). A larger proportion of pinto bean lines were intermediate (55\%) followed by pink (40\%), small red (33\%), black (23\%), navy ( $21 \%$ ) and great northern ( $16 \%$ ). There were no susceptible lines in the advanced lines of Middle-American market classes except navy bean (7\%).

Table 1.3. Disease reaction in advanced lines of the Middle-American genetic background in greenhouse condition.

| Market Class | Resistant $^{\mathrm{x}}$ | Intermediate $^{\mathrm{y}}$ | Susceptible $^{\mathrm{z}}$ | Total Lines |
| :--- | :--- | :--- | :--- | :--- |
| Pinto | 5 | 6 | 0 | 11 |
| Navy | 10 | 3 | 1 | 14 |
| Black | 23 | 7 | 0 | 30 |
| Great Northern | 16 | 3 | 0 | 19 |
| Small Red | 4 | 2 | 0 | 6 |
| Pink | 3 | 2 | 0 | 5 |
| Total | 61 | 23 | 1 | 85 |
| ". "x" |  |  |  |  |

Leaf CBB ratings (1-9) scale: " $x$ " represents the disease severity score ( $1-3=1-25.5 \%$ ), " $y$ " represents the disease severity score ( $>3-6=25.6-64.5 \%$ ) and " $z$ " represents the disease severity score ( $>6-9=64.6-100 \%$ ).

In contrast to advanced lines, preliminary pinto bean lines evaluated had a higher frequency of resistant (70\%) (Table 1.4). Preliminary small red (55\%) and great northern (51\%) were equal frequency of resistant lines. The frequency of resistant pink and black bean lines were ( $47 \%$ ) and ( $20 \%$ ) respectively. No preliminary navy bean lines were found to be resistant in the Middle-American market classes (Table 1.4). In the intermediate resistant group higher frequency was observed in the black bean lines (73\%) followed by navy (63\%), pink (53\%), small red (45\%), great northern (46\%) and pinto (28\%). The highest frequency of susceptible
was in navy bean lines (38\%) and no susceptible lines were small red and pink bean market classes.

Table 1.4. Disease reaction in preliminary lines of the Middle-American genetic background in greenhouse condition.

| Market Class $^{\text {Resistant }}{ }^{\mathrm{x}}$ | Intermediate $^{\mathrm{y}}$ | Susceptible $^{\mathrm{z}}$ | Total lines |  |
| :--- | :--- | :--- | :--- | :--- |
| Pinto | 122 | 48 | 1 | 171 |
| Navy | 0 | 25 | 15 | 40 |
| Black | 14 | 51 | 5 | 70 |
| Great Northern | 33 | 30 | 2 | 65 |
| Small Red | 33 | 27 | 0 | 60 |
| Pink | 9 | 10 | 0 | 19 |
| Total | 211 | 191 | 23 | 425 |

Leaf CBB ratings (1-9) scale: "x" represents the disease severity score (1-3 = 1-25.5\%), " $y$ " represents the disease severity score ( $>3-6=25.6-64.5 \%$ ) and " $z$ " represents the disease severity score (>6-9 $=64.6-100 \%$ ).

In the preliminary breeding lines of Andean genetic background, dark red kidney (54\%), white kidney ( $53 \%$ ) lines and only $32 \%$ of the light red kidney lines were resistant (Table 1.5). Conversely light red kidney bean showed the highest frequency (68\%) in the intermediate resistance group followed by white kidney ( $47 \%$ ) and dark red kidney (35\%). Only $11 \%$ dark red kidney bean lines displayed susceptible in the Andean market classes.

Table 1.5. Disease reaction in preliminary lines of the Andean genetic background in greenhouse condition.

| Market Class $^{\text {Resistant }^{\mathrm{x}}}$ | Intermediate $^{\mathrm{y}}$ | Susceptible $^{\mathrm{z}}$ | Total lines |  |
| :--- | :--- | :--- | :--- | :--- |
| Dark Red Kidney | 20 | 13 | 4 | 37 |
| Light Red Kidney | 10 | 21 | 0 | 31 |
| White Kidney | 8 | 7 | 0 | 15 |
| Total | 38 | 41 | 4 | 83 |

Leaf CBB ratings (1-9) scale: "x" represents the disease severity score ( $1-3=1-25.5 \%$ ), " $y$ " represents the disease severity score ( $>3-6=25.6-64.5 \%$ ) and " $z$ " represents the disease severity score ( $>6-9=64.6-100 \%$ ).

Genotypic analysis of CBB resistance. The SAP6 marker was present in 374 of 593
(63\%) advanced and preliminary NDSU breeding lines evaluated. The SU91 marker was present much less frequently, only 97 (17\%), and 61 lines ( $10 \%$ ), respectively, contained both markers.

Among the 85 advanced breeding lines belonging to the Middle-American gene pool, $100 \%$ of
small red, $80 \%$ of pink $73 \%$ of black, $71 \%$ of navy, $27 \%$ of pinto and $21 \%$ of great northern bean lines evaluated contained the SAP6 marker (Table 1.6). The SU91 marker was present in $67 \%$ of small red lines, $20 \%$ of pink and $7 \%$ of black bean lines evaluated. Both markers were observed in $67 \%$ of small red bean lines, $20 \%$ pink and $7 \%$ black bean lines.

Table 1.6. Marker amplification in advanced lines of Middle-American genetic background.

| Market class | No <br> marker $^{\mathrm{a}}$ | SAP6 <br> marker $^{\mathrm{b}}$ | SU91 <br> marker $^{\mathrm{c}}$ | SAP6/SU91 <br> markers $^{\mathrm{d}}$ | Total lines |
| :--- | :--- | :--- | :--- | :--- | :--- |
| Pinto | 8 | 3 | 0 | 0 | 11 |
| Navy | 4 | 10 | 0 | 0 | 14 |
| Black | 8 | 22 | 2 | 2 | 30 |
| Great Northern | 15 | 4 | 0 | 0 | 19 |
| Small red | 0 | 6 | 4 | 4 | 6 |
| Pink | 1 | 4 | 1 | 1 | 5 |
| Total | 36 | 49 | 7 | 7 | 85 |

Marker composition "a" represents do not contain SAP6 and/or SU91 marker, "b" represents contains SAP6 marker, "c" represents contain SU91 marker, "d" represents contain both SAP6/SU91 markers.

Among the 425 preliminary lines within the Middle-American genetic background, the SAP6 marker amplified in $98 \%$ of navy, $87 \%$ of black, $74 \%$ of pink, $72 \%$ of small red, $53 \%$ of pinto, and $51 \%$ of great northern bean lines (Table 1.7). Fewer preliminary lines possessed the SU91 marker, where $72 \%$ of small red, $32 \%$ of pink, $12 \%$ of pinto and $1 \%$ of black bean lines, no great northern or navy bean lines amplified with SU91. Both markers were observed in 48\% of small red, $16 \%$ of pink and $5 \%$ of pinto bean lines.

Table 1.7. Marker amplification in preliminary lines of Middle-American genetic background.

| Market class | No <br> marker $^{\mathrm{a}}$ | SAP6 <br> marker $^{\mathrm{b}}$ | SU91 <br> marker $^{\mathrm{c}}$ | SAP6/SU91 <br> markers $^{\mathrm{d}}$ | Total <br> lines |
| :--- | :--- | :--- | :--- | :--- | :--- |
| Pinto | 69 | 91 | 20 | 9 | 171 |
| Navy | 1 | 39 | 0 | 0 | 40 |
| Black | 9 | 61 | 1 | 1 | 70 |
| Great Northern | 32 | 33 | 0 | 0 | 65 |
| Small Red | 3 | 43 | 43 | 29 | 60 |
| Pink | 2 | 14 | 6 | 3 | 19 |
| Total | 116 | 281 | 70 | 42 | 425 |

Marker composition "a" represents do not contain SAP6 and/or SU91 marker, "b" represents contains SAP6 marker, "c" represents contain SU91 marker, "d" represents contain both SAP6/SU91 markers.

No advanced dry bean lines with an Andean genetic background were evaluated. Among the 83 Andean preliminary lines evaluated, the SAP6 marker was most commonly amplified in light red kidney beans (65\%), followed by white kidney (53\%) and dark red kidney (43\%) (Table 1.8). The SU91 marker amplified in $35 \%$ of dark red kidney lines followed by white kidney (20\%), and light red kidney (13\%). Both markers amplified in $22 \%$ of dark red kidney beans lines, followed by $10 \%$ of light red kidney, and $7 \%$ of white kidney bean lines.

Table 1.8. Marker amplification in preliminary lines of Andean genetic background.

| Market class | No <br> marker $^{\mathrm{a}}$ | SAP6 <br> marker $^{\mathrm{b}}$ | SU91 <br> marker $^{\mathrm{c}}$ | SAP6/SU91 <br> marker $^{\mathrm{d}}$ | Total <br> lines |
| :--- | :--- | :--- | :--- | :--- | :--- |
| Dark Red Kidney | 16 | 16 | 13 | 8 | 37 |
| Light Red Kidney | 10 | 20 | 4 | 3 | 31 |
| White Kidney | 5 | 8 | 3 | 1 | 15 |
| Total | 31 | 44 | 20 | 12 | 83 |

Marker composition "a" represents do not contain SAP6 and/or SU91 marker, "b" represents contains SAP6 marker, "c" represents contain SU91 marker, "d" represents contain both SAP6/SU91 markers.

Disease Severity in greenhouse experiments. The average percentage of CBB disease severity across all Middle American (24.9\%) and Andean (24.1\%) lines was not significantly different; therefore, market classes were not separated by genetic background when analyzing for disease severity. Average percentage of disease severity of individual market classes ranged from $42.6 \%$ for navy beans to $16.9 \%$ for pinto beans (Table 1.9). Navy beans were significantly
more susceptible than any other market class and pinto beans were significantly more resistant than all other market classes except for great northern.

Table 1.9. Comparison of common bacterial blight disease severity in percentage between dry bean market classes in greenhouse condition.

| Market Class | Disease Severity <br> (in $\%)^{\mathrm{x}}$ |  |
| :--- | :--- | :--- |
| Navy | 42.6 | a |
| Black | 27.0 | b |
| Pink | 23.1 | bcd |
| Small Red | 20.2 | cd |
| Great Northern | 19.5 | de |
| Pinto | 16.9 | e |
| Light Red Kidney | 24.5 | b |
| Dark Red Kidney | 24.2 | bcd |
| White Kidney | 23.6 | bcd |

"X" represents mean CBB leaf ratings in \% (evaluated 21 days after inoculation in the first fully expanded trifoliate leaf) in greenhouse. Leaf ratings were recorded on a (1-9) scale, where $1=$ no necrosis/chlorosis and $9=91-100 \%$ regions necrosis/chlorosis. The comparison of CBB severity among the market classes were done using Tukey-Kramer least square means separation test. Mean with same letter is not significantly different at ( $\mathrm{P}>0.05$ ) level.

## Evaluation of marker association with disease severity in greenhouse conditions. Genetic

background: In the Middle American genetic background, the mean disease severity in percentages across marker groups ranged from $16.6 \%$ to $26.1 \%$ (Table 1.10). The lines containing SU91 or both markers were significantly more resistant than the lines containing only SAP6, or neither marker. Lines containing SAP were significantly more susceptible than lines containing neither marker. In the Andean genetic background, the range of percentages of disease severity was slightly higher than that of the Middle-American lines, ranging from $12.8 \%$ to $30.4 \%$ across marker groups. The lines containing SU91 or both markers were significantly more resistant than the lines with only SAP6 or neither marker. Contrary to results from the

Middle American background, lines with SAP6 were significantly more resistant than lines containing neither marker.

Table 1.10. Association of markers with common bacterial blight disease severity in percentage within genetic backgrounds in greenhouse condition.

| Genetic Background | No <br> Marker $^{\mathrm{x}}$ | SAP6 <br> marker $^{\mathrm{y}}$ | SU91 <br> marker $^{\mathrm{z}}$ | Both <br> Markers $^{\mathrm{p}}$ |  |  |  |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| Middle American | 20.1 | b | 26.1 | a | 16.6 | c | 17.5 |
| c |  |  |  |  |  |  |  |
| Andean | 30.4 | a | 23.5 | b | 12.8 | c | 15.3 | c | cher |
| :--- |

Marker composition " $x$ " represents do not contain SAP6 and/or SU91 marker, " $y$ " represents contains SAP6 marker, " $z$ " represents contain SU91 marker, " $p$ " represents contain both SAP6/SU91 markers between the two genetic background. Mean CBB leaf ratings in \% (evaluated 21 days after inoculation in the first fully expanded trifoliate leaf) in greenhouse. Leaf ratings were recorded in $\%$ on a (1-9) scale, where $1=$ no necrosis/chlorosis and $9=91-100 \%$ necrotic/chlorotic regions. The association of markers with CBB severity in the Andean and Middle-American genetic background were done using Tukey-Kramer least square means separation test. Mean with same letter is not significantly different at ( $\mathrm{P}>0.05$ ) level.

Market Class: Mean CBB disease severity in percentage ranged from $12.2 \%$ to $20.0 \%$ in the pinto, $25.5 \%$ to $44.5 \%$ in the navy, $12.1 \%$ to $28.2 \%$ in the black, $18.9 \%$ to $20.1 \%$ in the great northern, $14.3 \%$ to $26.2 \%$ in the small red, and $15.1 \%$ to $30.0 \%$ in the pink market classes (Table 1.11). Within the pink and small red market classes, lines containing SU91 demonstrated a significantly better level of resistance than the lines with SAP6 or neither marker. Lines with both markers in the small red market class displayed significantly lower CBB disease severity than lines with SAP6 but not lines with neither marker. Lines with SAP6 in the small red market class displayed the highest level of disease incidence of the Middle-American market classes. In the pink bean market class, lines with SU91 or both markers demonstrated a significantly better resistance level compared to the lines with SAP6 or neither marker. Lines within the pinto bean market class exhibited contrary results, lines containing SU91 exhibited significantly lower level of resistance than lines with SAP6 and a similar level of resistance as those lines without either marker. Pinto lines with both markers displayed significantly lower CBB disease incidence than
lines in any other group. No lines within the black bean market class were identified with only the SU91 marker. Lines in the black bean market class with both markers had a significantly better level of CBB resistance compared to lines with only SAP6 or neither marker. There was no significant difference between lines with SAP6 or neither marker. SU91 was not identified in any navy or great northern lines. Both market classes displayed similar trends, with lines containing SAP6 having higher disease incidence but the difference was significant only in the navy market class.

Table 1.11. Association of markers with common bacterial blight mean disease severity in percentage within individual Middle American market classes in greenhouse condition.

| Market Class | No Marker $^{\mathrm{x}}$ |  | SAP6 marker $^{\mathrm{y}}$ |  | SU91 marker $^{\mathrm{z}}$ |  | Both Markers $^{\mathrm{p}}$ |  |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| Pinto | 18.7 | a | 15.9 | b | 20.0 | a | 12.2 | c |
| Navy | 25.5 | b | 44.5 | a | - |  | - |  |
| Black | 25.2 | a | 28.2 | a | - |  | 12.1 | b |
| Great Northern | 18.9 | a | 20.1 | a | - |  | - |  |
| Small Red | 25.1 | ab | 26.2 | a | 14.3 | c | 19.2 | b |
| Pink | 30.0 | a | 25.2 | a | 15.1 | b | 17.8 | b |

Marker composition " $x$ " represents do not contain SAP6 and/or SU91 marker, " $y$ " represents contains SAP6 marker, " $z$ " represents contain SU91 marker, " $p$ " represents contain both SAP6/SU91 markers in the Middle-American market classes. Mean CBB leaf ratings in \% (evaluated 21 days after inoculation in the first fully expanded trifoliate leaf) in greenhouse. Leaf ratings were recorded on a (1-9) scale, where $1=$ no necrosis/chlorosis and $9=91-100 \%$ necrotic/chlorosis regions. The association of markers with CBB severity in the market classes of Middle-American genetic background were done using Tukey-Kramer least square means separation test. Mean with same letter is not significantly different at ( $\mathrm{P}>0.05$ ) level.

In the Andean market classes, dark red, light red, and white kidney, all lines with no markers had either similar percentages of mean disease severity levels or significantly higher severity levels compared to lines with SAP6 (Table 1.12). In the dark red kidney market class, the mean CBB disease severity ranged from $5.4 \%$ to $31.9 \%$. Lines containing the SU91 marker demonstrated the best level of CBB resistance. This was significantly different from the lines containing SAP6 and the lines without either marker but not significantly different from lines
with both markers. In the light red kidney market classes, the lines with both markers had the lowest disease incidence, but this was only significantly different than the lines without either marker. CBB disease severity in the white kidney bean market class ranged from $12.6 \%$ to
26.7\%. Lines with SAP6, or neither marker, had significantly higher mean disease severity than lines containing SU91. No significant difference was observed between white kidney lines with both markers or SU91 alone.

Table 1.12. Association of markers with common bacterial blight mean disease severity in percentages within Andean market classes in greenhouse condition.

| Market Class | No Marker ${ }^{\text {x }}$ |  | SAP6 marker ${ }^{\text {y }}$ |  | SU91 marker ${ }^{2}$ |  | Both Markers ${ }^{\text {p }}$ |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Dark Red Kidney | 31.5 | a | 16.9 | b | 5.4 | c | 12.9 | bc |
| Light Red Kidney | 27.8 | a | 23.7 | ab | 25.1 | ab | 16.5 | b |
| White Kidney | 26.7 | a | 26.3 | a | 12.6 | b | 13.1 | ab |

Marker composition "x" represents do not contain SAP6 and/or SU91 marker, " $y$ " represents contains SAP6 marker, " $z$ " represents contain SU91 marker, " $p$ " represents contain both SAP6/SU91 markers in the Andean market classes. Mean CBB leaf ratings in \% (evaluated 21 days after inoculation in the first fully expanded trifoliate leaf) in greenhouse. Leaf ratings were recorded on a (1-9) scale, where $1=$ no necrosis/chlorosis and $9=91-100 \%$ necrotic/chlorosis regions. The association of markers with CBB severity in the market classes of Andean genetic background were done using Tukey-Kramer least square means separation test. Mean with same letter is not significantly different at $(\mathrm{P}>0.05)$ level.

The genotyping with SAP6 and SU91 suggests CBB resistance would be expected in over $70 \%$ of the advanced lines, which was very similar to the observed phenotyping results. Genotyping of the preliminary lines suggested over $90 \%$ would exhibit resistance. This result was not similar to the phenotyping result of less than $50 \%$ of the lines displaying resistance. However, in our study in the Middle-American genetic background nine advanced, seven preliminary lines and in the Andean genetic background only three preliminary lines were identified as best resistant to CBB (Table 1.13).

Table 1.13. The best lines (CBB score: 1-2) of CBB resistant among the advanced and preliminary lines of Middle-American and Andean market classes.

| Middle-American |  |  | Andean |  |
| :---: | :---: | :---: | :---: | :---: |
| Market class | Advanced lines | Preliminary lines | Market class | Preliminary lines |
| Black | Zorro, ND071244 | - | - | - |
| Great Northern | ND09734, ND112823, ND112844 | - | Dark Red Kidney | 2026 DRK, 2053 DRK, 2114 DRK |
| Small Red | ND080509, ND080547, NDF09107 | ND121928 | - | - |
| Navy | NDF09202 | - | - | - |
| Pinto | - | ND121334, <br> ND121443, <br> ND121446, <br> ND121450, <br> ND121454, <br> ND121478 | - | - |
| Total | 9 | 7 |  | 3 |
| Total | 19 |  |  |  |

Disease severity in field experiments. In the field experiment the percentages of mean disease severity of individual market classes ranged from $19.8 \%$ to $55.4 \%$ in the small red and navy bean market class, respectively (Table 1.14). Navy beans were significantly different in percentages mean disease severity with intermediate resistant than any other market classes and small red beans were more resistant than any other market classes. All market classes displayed intermediate level of disease severity except small red which showed resistant reaction.

Table 1.14. Comparison of common bacterial blight disease severity in percentages between $\underline{\text { market classes in the field conditions. }}$

| Market Class | Disease Severity $^{\mathrm{x}}$ (in \%) |
| :--- | :--- |
| Navy | 55.4 a |
| Great Northern | 51.0 a |
| Black | 50.6 a |
| Pinto | 43.8 b |
| Pink | 37.7 b |
| Small Red | 19.8 c |

" x " represents mean CBB leaf ratings (evaluated based on natural infection in the field of Prosper, August, 2014). Leaf ratings were recorded on a (1-9) scale, where $1=$ no necrosis/chlorosis and $9=91-100 \%$ necrotic/chlorosis regions. The comparison of CBB severity among the market classes were done using Tukey-Kramer least square means separation test. Mean with same letter is not significantly different at ( $\mathrm{P}>0.05$ ) level.

## Evaluation of marker association with disease severity in field conditions. Market class:

In the Middle-American genetic background, the mean disease severity across marker groups ranged from $39.1 \%$ to $51.8 \%$ in pinto, $51.8 \%$ to $55.7 \%$ in navy, $50.3 \%$ to $52.6 \%$ in black, $50.5 \%$ to $52.3 \%$ in great northern, $13.0 \%$ to $25.9 \%$ in small red and $25.8 \%$ to $43.1 \%$ in the pink market classes (Table 1.15). Within the small red and pink bean market classes, the lines containing SU91 showed better level of resistance than the lines with SAP6 or both markers but they were not significantly different across the marker group. Lines with SAP6 in pink and small red market class displayed the highest level of disease incidence. Lines within the pinto bean market class displayed contrary result, the SU91 containing lines exhibited better level of intermediate resistance than any other marker combinations but the SU91 containing lines showed the highest level of disease incidence. Similar level of disease incidence was observed in the no marker and SU91 containing liens. In the navy, black and great northern market classes there were no significant difference in the percentages of mean disease severity with SAP6 and no marker containing lines. In this market classes SU91 and both markers were not identified. Importantly,
it was observed that there was no significant difference in percentages of mean disease severity within marker groups in each market class of the Middle-American genetic background during field evaluation.

Table 1.15. Association of markers with common bacterial blight mean disease severity in percentages within individual Middle-American market classes in field conditions.

| Market Class | No <br> Marker ${ }^{\mathrm{X}}$ | SAP6 Marker ${ }^{\text {y }}$ | SU91 Marker ${ }^{\text {z }}$ | Both Markers ${ }^{\text {p }}$ |
| :---: | :---: | :---: | :---: | :---: |
| Pinto | 49.3 a | 39.1 a | 51.8 a | 43.1 a |
| Navy | 51.8 a | 55.7 a | - | - |
| Black | 52.6 a | 50.3 a | - | - |
| Great <br> Northern | 50.5 a | 52.3 a | - | - |
| Small Red | - | 25.9 a | 13.0 a | 20.0 a |
| Pink | - | 43.1 a | 25.8 a | 35.5 a |

Marker composition "x" represents do not contain SAP6 and/or SU91 marker, " $y$ " represents contains SAP6 marker, " $z$ " represents contain SU91 marker, " $p$ " represents contain both SAP6/SU91 markers in the Middle-American market classes. Mean CBB leaf rating in \% (evaluated based on natural infection in the field of Prosper, August, 2014) in field. Leaf ratings were recorded on a (1-9) scale, where $1=$ no necrosis/chlorosis and $9=91-100 \%$ necrotic/chlorosis regions. Mean with same letter is not significantly different at ( $\mathrm{P}>0.05$ ) level.

Comparison of CBB disease severity between greenhouse and field conditions. The overall correlation of CBB disease severity between greenhouse and field were significant with a weak correlation where $\mathrm{r}=0.16(\mathrm{p} \leq 0.05)$ (Table 1.16). Within market classes only great northern market class showed significant correlation between greenhouse and field disease severity with $\mathrm{r}=0.38(\mathrm{p} \leq 0.05)$ (Table 1.16). The other market classes did not exhibit significant correlation between field and greenhouse disease severity except navy which displayed a negative correlation.

Table 1.16. Phenotypic correlation between greenhouse and field conditions among the Middle-American market classes with 188 advanced and preliminary lines.

Field

|  | Overall | Pinto | Navy | Black | Small Red | Great Northern | Pink |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| Greenhouse | 0.16 | 0.07 | -0.26 | 0.15 | 0.13 | 0.38 | $0.77^{\mathrm{a}}$ |
|  | 0.0262 | 0.6158 | 0.3388 | 0.2499 | 0.6604 | 0.0224 | $0.0741^{\mathrm{b}}$ |

"a" represents $r$ value and "b" represents $p$ value.
In the greenhouse, most of the lines were resistant (110 lines), 70 lines displayed intermediate resistant and 8 lines showed susceptible whereas in the field, only 23 lines were found as resistant, 128 lines showed intermediate resistant and 37 lines found as susceptible among the 188 lines of different market classes (Table 1.17).

Table 1.17. Comparison of common bacterial blight disease resistant, intermediate and susceptible lines across market classes under greenhouse and field conditions.

| Field evaluations |  |  |  | Greenhouse evaluations |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Market class | Resistant ${ }^{\text {x }}$ | Intermediate ${ }^{\text {y }}$ | Susceptible ${ }^{\text {z }}$ | Resistant ${ }^{\text {x }}$ | Intermediate ${ }^{\text {y }}$ | Susceptible ${ }^{\text {z }}$ |
| Pinto | 9 | 38 | 7 | 41 | 13 | 0 |
| Navy | 0 | 12 | 4 | 5 | 6 | 5 |
| Black | 4 | 43 | 15 | 28 | 31 | 3 |
| Small |  |  |  |  |  |  |
| Red | 10 | 4 | 0 | 6 | 8 | 0 |
| Great |  |  |  |  |  |  |
| Northern | 0 | 25 | 11 | 27 | 9 | 0 |
| Pink | 0 | 6 | 0 | 3 | 3 | 0 |
| Total | 23 | 128 | 37 | 110 | 70 | 8 |
| Total |  |  |  |  |  | 188 |

Leaf CBB ratings (1-9) scale: " $x$ " represents the disease severity score ( $1-3=1-25.5 \%$ ), " $y$ " represents the disease severity score ( $>3-6=25.6-64.5 \%$ ) and " $z$ " represents the disease severity score (>6-9 $=64.6-100 \%$ ).

## Discussion

## Disease severity across the Andean and Middle-American market classes and association

 of markers to CBB resistance. CBB resistance in dry bean is a quantitative trait which exhibits low to moderate heritability. Here, a combined screening (genotypic and phenotypic) was done using 593 lines ( 85 advance lines and 508 preliminary lines) to evaluate their CBB resistance.Phenotypic selection has been shown to maintain minor effect QTL and help in selecting epistatic interactions that contribute to improved CBB resistance, while genotyping helps maintain the larger effect QTL (Miklas et al., 2006a; Mutlu et al., 2008). Evaluating dry bean lines using only molecular markers does not always result in lines with superior CBB resistance, as minor genes/QTL contributing to CBB resistance are lost during evaluation (Fourie, 2002). Thus, the combined use of molecular markers and phenotypic screening are the best way in developing CBB resistant dry bean lines. The studies performed here are the first to evaluate the relationship between the SCAR markers SU91 and SAP6 and their effectiveness in identifying CBB resistance over numerous dry bean market classes.

CBB disease severity of the susceptible check was susceptible to moderate (CBB disease score ranging from $45 \%, 58 \%, 71 \%, 84 \%$ and $96 \%$ ), possibly resulting from isolate selection, inoculation methods used or environmental conditions. Greenhouse trials were conducted in two greenhouse rooms that varied in size, across several months. Temperature and humidity are major contributing factors in the development of CBB and are more difficult to control in the larger room compared to the smaller room. Inoculum production and inoculation technique can also affect CBB development. Disease rating was conducted at 14 and 21 dpi, but a significant difference in CBB disease severity at 14 dpi was not observed; therefore, only the 21 dpi data was further analyzed. This agrees with the previous evaluations (Duncan et al., 2012; Vandemark et al., 2009). CBB was rated at $7,10,14$, and 21 dpi ; however, disease severity between resistant and susceptible lines was only distinguishable at 21 dpi .

CBB resistant lines were identified in each market class by phenotyping and/or genotyping. More than half of the advanced lines for each market class evaluated displayed phenotypic resistance. Within each market class, nearly half or more than half of the preliminary
lines demonstrated phenotypic resistance with the exception of light red kidney, where only a third of the lines displayed resistance, and navy, where none of the lines displayed resistance. The lack of CBB resistance found within the preliminary navy bean lines evaluated may become problematic if this trend continues in the early breeding pipeline. Navy beans rank second in production in North Dakota behind pinto beans. CBB resistance is present throughout the breeding pipeline in the remainder of the market classes, a positive finding for future dry bean releases with CBB resistance.

Previous research has indicated that the Middle-American genetic background possesses favorable QTL imparting higher level of resistance to CBB and, overall, Andean beans are more susceptible to CBB (Duncan et al., 2011; Miklas et al., 2011). However, in this study, no significant difference in CBB disease severity between the Andean genetic background and Middle-American genetic background was observed across all market classes. Within the market classes of the Andean genetic background, no significant differences were observed; however there were significant differences among market classes in the Middle American genetic background. This has been reported in previous research, where navy bean germplasm lines were more susceptible than pinto and black bean lines (Boersma et al., 2014). This confirms the results of more genetic diversity among the Middle American gene pool and less diversity among the Andean gene pool and suggests that differences observed between Middle American and Andean populations may depend on the market classes or genotypes being compared.

Resistance to CBB in dry bean is a quantitative trait, and partial resistance to CBB conferred by a single QTL. Pyramiding multiple QTL in a single genetic background helps to develop breeding lines from various resistance sources. It can help to increase the levels of partial resistance beyond that conferred by a single QTL (Nodari et al., 1993). O'Boyle and

Kelly, (2007) also reported that incorporation of multiple QTL enhance the higher level of resistance than conferred by a single QTL.

CBB disease severity between the Andean lines grouped by marker presence suggests an additive effect of the two marker linked QTL since lines were more resistant when both marker were present. Contrary to the Andean results, the two marker linked QTL do not appear to be additive in the Middle-American background. Rather, the presence of SAP6 appears to be linked with susceptibility and SU91 linked with resistance. In this host population, CBB disease severity was significantly lower when SU91 was present.

Our findings are consistent with previous studies indicating that both markers were effectively identify CBB resistance, depending on market class or population. Mutlu et al., (2005) found that presence of SAP6 and SU91 in pinto bean line ABCP-8 showed a high level of CBB resistance. O'Boyle et al., (2007) reported that breeding line VAX 5 containing SAP6 and SU91 markers displayed a CBB resistant greenhouse reaction. Our results are also supported by Duncan et al., (2012), where he conducted experiments in greenhouse and in field to evaluate the response of four parents DRK 1, DRK 2, Wilkinson 2 and VAX 3, which contain SAP6 and SU91. SAP6 and SU91 conferred very high levels of CBB resistance when incorporated into VAX 3 and VAX 6 breeding lines through direct disease resistance selection (Duncan et al., 2012). Line USDK-CBB-15 also has a high level of CBB resistance conferred from the presence of these two markers (Miklas et al., 2006b).

On the contrary, Duncan et al., (2011), also reported considerable variability of CBB response in genotypes with SAP6 and SU91. Viteri et al., (2014a), reported that black bean breeding line VAX 5 displayed an intermediate level of resistance. Miklas et al., (2006b), reported white kidney bean line USWK-CBB-17, also contains both SAP6 and SU91 markers
but it also exhibited intermediate level of CBB resistance. These conflicting results concerning the effect of the combination of SU91 and SAP6 our consistent with results from this study where the combination of both SAP6/SU91 markers provided a higher level of CBB resistance when compared to a single or neither marker in some market classes, but not others.

Across lines evaluated in this study from all major dry bean market classes grown in the U.S., SU91 was generally more effective than SAP6 in identifying lines with CBB resistance. The presence of SU91 conferred resistance to CBB in small red, pink, dark red kidney, light red kidney and white kidney. Duncan et al., (2012), also indicated that SU91 marker has a tighter association with the QTL to CBB resistance. Kelly et al., (2012), reported pink bean Rosetta, which possesses SU91, displaying CBB resistance under field conditions.

In this research study, pinto bean genotypes containing SAP6 showed high levels of CBB resistance. These results are consistent with Miklas et al. (2003), where a tight association of SAP6 marker with CBB resistance was observed. Vandemark et al., (2009), reported that SAP6 was linked to resistance in some genotypes but it was also found in susceptible genotypes. The lack of effect of SAP6 to CBB resistance in some genotypes could be due to recombination between the SAP6 marker and the QTL conditioning resistance (Vandemark et al., 2009). Genotypes containing neither of these markers also exhibited high levels of CBB resistance in some lines. Duncan et al., (2012), found that some dry bean lines displayed resistance but did not contain SAP6 or SU91. These results may be explained based on the populations used in the research, as well as the presence of unidentified CBB resistant QTL within the dry bean genome. In our study no correlation was observed between the CBB resistant genotypes and the presence of markers. So, these results imply that there were not resistant genotype with markers because some lacked markers genotype also showed resistant and some genotypes had marker but they
were not resistant. This findings were also supported by Yu et al., (2000), she reported that the number of resistant plant was much lower than the number of genotypes with SCAR markers. This is due to either a gametic or a zygotic selection pressure against CBB resistance or an unknown genetic factor which is tightly linked to the QTL for CBB resistance, considering under negative selection pressure.

Comparison of CBB reaction under greenhouse and field conditions. The overall CBB disease severity in the greenhouse and field was not consistent. In the field conditions only 23 lines were rated resistant, whereas, under greenhouse conditions, 110 lines were found as resistant. Our results disagree with the previous results where it was observed that in the field condition the CBB severity is less than greenhouse conditions (Duncan et al., 2012; Mutlu et al., 2005; O'Boyle et al., 2007; Osdaghi et al., 2010). This could be due to the aggressiveness of the pathogen in the field environment in 2014 in Prosper. There was no significant difference in effectiveness of markers to CBB resistance between lines containing markers in each market class. Only in the small red market class the SU91 and both markers combination were effective in predicting CBB resistance. Our findings are partially supported by Duncan et al., (2012) where small red beans were as resistant to CBB under both field (10 lines) and greenhouse (6 lines) conditions. In other market classes all marker combination was associated with intermediate level of resistance, which is also consistent with Viteri et al., (2014a) where he reported SAP6 and SU91 both or either marker were associated with resistant and intermediate levels of resistant. The expression of these QTL is highly influenced by environment and pathogen aggressiveness, so the lines of different market classes except small red evaluated in the field of Prosper exhibited intermediate level of resistance.

In summary, a total of 593 advance and preliminary NDSU breeding lines of different market classes belonging to the Andean and Middle-American genetic backgrounds were evaluated through genotyping and phenotyping against a virulent strain of Xap in the greenhouse. After screening the genotypes, 310 genotypes were found to be resistant, 255 genotypes were intermediate and only 28 genotypes were found to be susceptible under greenhouse conditions. One hundred-eighty eight advanced and preliminary lines were evaluated under field conditions and 23 lines were resistant, 128 lines were intermediate and 37 were susceptible. In the greenhouse conditions pinto beans were highly resistant and small red were highly resistant in the field condition. SU91 and both markers (SAP6/SU91) were more effective than the SAP6 marker at identifying lines with CBB resistance. However, previous results suggest that the role of specific QTL and their interaction between QTL to CBB resistance could be enhanced by the genotypes used and the isolates of Xap used for inoculation (Vandemark et al., 2009). Overall, this data suggests the effect of these two QTL depends on genetic background. CBB resistance cannot be ensured to be present based solely on the detection of markers in a plant genome. This relationship within market class should be further examined and going forward, care should be taken when interpreting genotypic data across market classes in the absence of accurate phenotypic data.

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# APPENDIX A. SUMMARY OF STATISTICAL ANALYSIS FOR COMMON BACTERIAL BLIGHT (CBB) AND ASSESSMENT OF SCAR MARKER EFFECTIVENESS ACROSS MARKET CLASSES OF ADVANCED AND PRELIMINARY BREEDING LINES OF ANDEAN AND MIDDLE-AMERICAN GENETIC BACKGROUND 

Table A.1. Analysis of variance of disease severity to common bacterial blight (CBB) of the different market classes of advanced and preliminary breeding lines of Andean and MiddleAmerican genetic background during greenhouse study.

| Source of variation | Degrees of <br> freedom | Mean <br> Square | F Value | P Value |
| :--- | :--- | :--- | :--- | :--- |
| Trial | 2 | 7754.5037 | 27.80 | $<.0001$ |
| Rep (Trial) | 6 | 545.7061 | 1.96 | 0.0683 |
| Genetic background | 1 | 224.6114 | 0.81 | 0.3696 |
| Market class (Genetic background) | 7 | 32070.9672 | 114.98 | $<.0001$ |

Table A.2. Analysis of variance of SCAR markers (SAP6/SU91) association to common bacterial blight (CBB) of the different market classes of advanced and preliminary breeding lines of Middle-American genetic background during greenhouse study.

| Source of variation | Degrees of <br> freedom | Mean <br> Square | F Value | P Value |
| :--- | :--- | :--- | :--- | :--- | :--- |
| Trial | 2 | 7526.80830 | 23.73 | $<.0001$ |
| Rep (Trial) | 6 | 652.76942 | 2.06 | 0.0549 |
| Marker | 3 | 16124.14136 | 50.84 | $<.0001$ |

Table A.3. Analysis of variance of SCAR markers (SAP6/SU91) association to common bacterial blight (CBB) of the different market classes of preliminary breeding lines within Andean genetic background during greenhouse study.

| Source of variation | Degrees of <br> freedom | Mean <br> Square | F Value | P Value |
| :--- | :--- | :--- | :--- | :--- |
| Trial | 2 | 681.30698 | 2.17 | 0.1158 |
| Rep (Trial) | 6 | 86.04622 | 0.27 | 0.9492 |
| Marker | 3 | 5760.47793 | 18.31 | $<.0001$ |

Table A.4. Analysis of variance of SCAR markers (SAP6/SU91) association to common bacterial blight (CBB) of black bean market class advanced and preliminary breeding lines of the Middle-American genetic background during greenhouse study.

| Source of variation | Degrees of <br> freedom | Mean Square | F Value | P Value |
| :--- | :--- | :--- | :--- | :--- |
| Trial | 2 | 4622.527546 | 13.03 | $<.0001$ |
| Rep (Trial) | 6 | 224.703534 | 0.63 | 0.7035 |
| Marker | 2 | 3278.368208 | 9.24 | 0.0001 |

Table A.5. Analysis of variance of SCAR marker (SAP6) association to common bacterial blight (CBB) of great northern bean market class advanced and preliminary breeding lines of the Middle-American genetic background during greenhouse study.

| Source of variation | Degrees of <br> freedom | Mean <br> Square | F Value | P Value |
| :--- | :--- | :--- | :--- | :--- |
| Trial | 2 | 1519.536613 | 6.89 | 0.0011 |
| Rep (Trial) | 6 | 81.181904 | 0.37 | 0.8992 |
| Marker | 1 | 216.584081 | 0.98 | 0.3221 |

Table A.6. Analysis of variance of SCAR markers (SAP6) association to common bacterial blight (CBB) of navy bean market class advanced and preliminary breeding lines of the MiddleAmerican genetic background during greenhouse study.

| Source of variation | Degrees of <br> freedom | Mean <br> Square | F Value | P Value |
| :--- | :--- | :--- | :--- | :--- |
| Trial | 2 | 831.37092 | 1.27 | 0.2821 |
| Rep (Trial) | 6 | 210.32841 | 0.32 | 0.9258 |
| Marker | 1 | 11878.49831 | 18.14 | $<.0001$ |

Table A.7. Analysis of variance of SCAR markers (SAP6/SU91) association to common bacterial blight (CBB) of pink bean market class of advanced and preliminary breeding lines of the Middle-American genetic background during greenhouse study.

| Source of variation | Degrees of <br> freedom | Mean <br> Square | F Value | P Value |
| :--- | :--- | :--- | :--- | :--- | :--- |
| Trial | 2 | 2655.922012 | 14.10 | $<.0001$ |
| Rep (Trial) | 6 | 57.542136 | 0.31 | 0.9334 |
| Marker | 3 | 1347.537721 | 7.16 | 0.0001 |

Table A.8. Analysis of variance of SCAR markers (SAP6/SU91) association to common bacterial blight (CBB) of pinto bean market class of advanced and preliminary breeding lines of the Middle-American genetic background during greenhouse study.

| Source of variation | Degrees of <br> freedom | Mean <br> Square | F Value | P Value |
| :--- | :--- | :--- | :--- | :--- |
| Trial | 2 | 140.273339 | 1.01 | 0.3634 |
| Rep (Trial) | 6 | 180.317531 | 1.30 | 0.2530 |
| Marker | 3 | 1487.327178 | 10.74 | $<.0001$ |

Table A.9. Analysis of variance of SCAR markers (SAP6/SU91) association to common bacterial blight (CBB) of small red bean market class of advanced and preliminary breeding lines of the Middle-American genetic background during greenhouse study.

| Source of variation | Degrees of <br> freedom | Mean <br> Square | F Value | P Value |
| :--- | :--- | :--- | :--- | :--- |
| Trial | 2 | 2384.685464 | 12.20 | $<.0001$ |
| Rep (Trial) | 6 | 251.819039 | 1.29 | 0.2607 |
| Marker | 3 | 3025.336554 | 15.48 | $<.0001$ |

Table A.10. Analysis of variance of SCAR markers (SAP6/SU91) association to common bacterial blight (CBB) of dark red kidney bean market class of preliminary breeding lines of the Andean genetic background during greenhouse study.

| Source of variation | Degrees of <br> freedom | Mean <br> Square | F Value | P Value |
| :--- | :--- | :--- | :--- | :--- |
| Trial | 2 | 615.82651 | 1.52 | 0.2214 |
| Rep (Trial) | 6 | 255.35610 | 0.63 | 0.7054 |
| Marker | 3 | 5775.83606 | 14.27 | $<.0001$ |

Table A.11. Analysis of variance of SCAR markers (SAP6/SU91) association to common bacterial blight (CBB) of light red kidney bean market class of preliminary breeding lines of the Andean genetic background during greenhouse study.

| Source of variation | Degrees of <br> freedom | Mean <br> Square | F Value | P Value |
| :--- | :--- | :--- | :--- | :--- |
| Trial | 2 | 212.816441 | 0.85 | 0.4308 |
| Rep (Trial) | 6 | 182.129205 | 0.72 | 0.6309 |
| Marker | 3 | 608.534445 | 2.42 | 0.0675 |

Table A.12. Analysis of variance of SCAR markers (SAP6/SU91) association to common bacterial blight (CBB) of white kidney bean market class of preliminary breeding lines of the Andean genetic background during greenhouse study.

| Source of variation | Degrees of <br> freedom | Mean <br> Square | F Value | P Value |
| :--- | :--- | :--- | :--- | :--- |
| Trial | 2 | 178.085221 | 0.66 | 0.5184 |
| Rep (Trial) | 6 | 123.296619 | 0.46 | 0.8367 |
| Marker | 3 | 888.377971 | 3.31 | 0.0244 |

Table A.13. Analysis of variance of disease severity to common bacterial blight (CBB) of the different market classes of advanced and preliminary breeding lines of Middle-American genetic background during field study.

| Source of variation | Degrees of <br> Freedom | Mean <br> Square | F Value | P Value |
| :--- | :--- | :--- | :--- | :--- |
| Rep | 1 | 71.09641 | 0.22 | 0.6409 |
| Market Class | 5 | 5556.36804 | 17.03 | $<.0001$ |

Table A.14. Analysis of variance of SCAR markers (SAP6/SU91) association to common bacterial blight (CBB) of black bean market class advanced and preliminary breeding lines of the Middle-American genetic background during field study.

| Source of variation | Degrees of <br> Freedom | Mean <br> Square | F Value | P Value |
| :--- | :--- | :--- | :--- | :--- |
|  |  |  |  |  |
| Rep | 1 | 1140.129032 | 3.33 | 0.0703 |
| Marker | 1 | 68.673910 | 0.20 | 0.6548 |

Table A.15. Analysis of variance of SCAR markers (SAP6/SU91) association to common bacterial blight (CBB) of great northern bean market class advanced and preliminary breeding lines of the Middle-American genetic background during field study.

| Source of variation | Degrees of <br> Freedom | Mean <br> Square | F Value | P Value |
| :--- | :--- | :--- | :--- | :--- |
| Rep | 1 | 760.5000000 | 2.34 | 0.1310 |
| Marker | 1 | 54.6262626 | 0.17 | 0.6834 |

Table A.16. Analysis of variance of SCAR markers (SAP6/SU91) association to common bacterial blight (CBB) of navy bean market class advanced and preliminary breeding lines of the Middle-American genetic background during field study.

| Source of variation | Degrees of <br> Freedom | Mean <br> Square | F Value | P Value |
| :--- | :--- | :--- | :--- | :--- | :--- |
| Rep | 1 | 132.0312500 | 1.01 | 0.3241 |
| Marker | 1 | 28.5187500 | 0.22 | 0.6446 |

Table A.17. Analysis of variance of SCAR markers (SAP6/SU91) association to common bacterial blight (CBB) of pink bean market class advanced and preliminary breeding lines of the Middle-American genetic background during field study.

| Source of variation | Degrees of <br> Freedom | Mean <br> Square | F Value | P Value |
| :--- | :--- | :--- | :--- | :--- |
| Rep | 1 | 352.083333 | 2.78 | 0.1341 |
| Marker | 2 | 239.416666 | 1.89 | 0.2129 |

Table A.18. Analysis of variance of SCAR markers (SAP6/SU91) association to common bacterial blight (CBB) of pinto bean market class advanced and preliminary breeding lines of the Middle-American genetic background during field study.

| Source of variation | Degrees of <br> Freedom | Mean <br> Square | F Value | P Value |
| :--- | :--- | :--- | :--- | :--- |
| Rep | 1 | 551.259259 | 1.35 | 0.2478 |
| Marker | 3 | 922.569444 | 2.26 | 0.0858 |

Table A.19. Analysis of variance of SCAR markers (SAP6/SU91) association to common bacterial blight (CBB) of small red bean market class advanced and preliminary breeding lines of the Middle-American genetic background during field study.

| Source of variation | Degrees of <br> Freedom | Mean <br> Square | F Value | P Value |
| :--- | :--- | :--- | :--- | :--- |
| Rep | 1 | 135.0803571 | 1.09 | 0.3075 |
| Marker | 2 | 166.6696429 | 1.34 | 0.2803 |

APPENDIX B. PHENOTYPIC DATA OF COMMON BACTERIAL BLIGHT DISEASE SEVERITY IN GREENHOUSE CONDITION AND THE GENOTYPIC DATA


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| ID code | Class | 1st trial |  |  |  |  |  |  |  |  |  | 2nd trial |  |  |  |  |  |  |  |  |  | 3rd trial |  |  |  |  |  |  |  |  |  | Marker data |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | Plant 1 |  |  | Plant 2 |  |  | Plant 3 |  |  | Mean | Plant 1 |  |  | Plant 2 |  |  | Plant 3 |  |  | Mean | Plant 1 |  |  | Plant 2 |  |  | Plant 3 |  |  | Mean |  |  |  |
|  |  | L1 | L2 | L3 | L1 | L2 | L3 | L1 | L2 | L3 |  | L1 | L2 | L3 | L1 | L2 | L3 | L1 | L2 | L3 |  | L1 | L2 | L3 | L1 | L2 | L3 | L1 | L2 | L3 |  | SAP6 | SU91 | Both |
| ND070612 | Navy | 5 | 5 | 5 | 5 | 6 | 6 | 6 | 6 | 6 | 5.6 | 7 | 7 | 7 | 5 | 5 | 5 | 7 | 7 | 7 | 6.3 | 6 | 6 | 6 | 7 | 7 | 7 | . | . | . | 6.5 | 0 | 0 | 0 |
| ND070717 | Navy | 3 | 3 | 3 | 4 | 5 | 5 | 5 | 7 | 7 | 4.7 | 2 | 2 | 2 | 2 | 3 | 4 | . | . | . | 2.5 | 2 | 2 | 2 | 2 | 3 | 3 | 2 | 2 | 3 | 2.3 | 1 | 0 | 1 |
| ND080742 | Navy | 2 | 2 | 3 | 3 | 3 | 4 | 2 | 3 | 3 | 2.8 | 3 | 3 | 3 | 2 | 3 | 2 | 2 | 3 | 3 | 2.7 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 0 | 1 |
| ND080788 | Navy | 2 | 2 | 2 | 2 | 2 | 3 | 2 | 2 | 3 | 2.2 | 3 | 4 | 6 | 2 | 2 | 2 | 2 | 2 | 2 | 2.8 | 2 | 2 | 2 | 4 | 4 | 4 | 4 | 3 | 3 | 3.1 | 1 | 0 | 1 |
| ND080805 | Navy | 2 | 3 | 3 | 3 | 3 | 4 | 2 | 3 | 3 | 2.9 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 3 | 3 | 3 | 3 | 3 | 3 | . | . | . | 3 | 1 | 0 | 1 |
| ND080910 | Navy | 2 | 2 | 2 | 3 | 3 | 4 | 2 | 3 | 3 | 2.7 | 2 | 3 | 3 | 2 | 2 | 2 | 2 | 2 | 2 | 2.2 | 3 | 3 | 3 | 2 | 2 | 3 | 2 | 2 | 2 | 2.4 | 1 | 0 | 1 |
| NDF09201 | Navy | 3 | 3 | 4 | 2 | 3 | 3 | . | . | . | 3 | 3 | 3 | 3 | 2 | 3 | 3 | . | . | . | 2.8 | 2 | 2 | 2 | 4 | 4 | 4 | 2 | 2 | 2 | 2.7 | 1 | 0 | 1 |
| NDF09202 | Navy | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 1 | . | 1.8 | 2 | 2 | 2 | 2 | 2 | 2 | . | . | . | 2 | 2 | 2 | 2 | 2 | 2 | 2 | . | . | . | 2 | 0 | 0 | 0 |
| ZORRO | Black | 2 | 2 | 2 | 2 | 2 | 2 | . | . | . | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | . | . | . | 2 | 1 | 0 | 1 |
| ND060613 | Black | 2 | 2 | 2 | 2 | 2 | 2 | 3 | 3 | 3 | 2.3 | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 2 | 3 | 2.8 | 2 | 2 | 3 | 2 | 3 | 3 | 2 | 2 | 2 | 2.3 | 1 | 0 | 1 |
| ND060769 | Black | 2 | 2 | 2 | 2 | 2 | 3 | . | . | . | 2.2 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 0 | 0 | 0 |
| ND071065 | Black | 3 | 4 | 6 | 4 | 4 | . | 2 | 3 | 3 | 3.6 | 2 | 2 | 2 | 4 | 6 | 6 | . | . | . | 3.7 | 2 | 2 | 2 | 2 | 2 | 3 | 2 | 2 | 3 | 2.2 | 1 | 0 | 1 |
| ND071089 | Black | 3 | 3 | 2 | 2 | 2 | 2 | 4 | 5 | 5 | 3.1 | 2 | 3 | 3 | 2 | 2 | 2 | 2 | 2 | 3 | 2.3 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 0 | 1 |
| ND071206 | Black | 2 | 2 | 3 | 3 | 3 | 3 | 2 | 3 | 3 | 2.7 | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 2 | 3 | 2.8 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 0 | 1 |
| ND071230 | Black | 3 | 3 | 4 | 2 | 2 | 3 | 2 | 2 | 2 | 2.6 | 3 | 4 | 4 | 3 | 3 | 4 | 3 | 3 | 3 | 3.3 | 2 | 2 | 3 | 2 | 3 | 3 | 2 | 3 | 3 | 2.6 | 1 | 0 | 1 |
| ND071244 | Black | 2 | 2 | 2 | 2 | 2 | 3 | 2 | 2 | 2 | 2.1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 0 | 0 | 0 |
| ND071249 | Black | 2 | 2 | 2 | 2 | 3 | 3 | 3 | 3 | 3 | 2.6 | 3 | 3 | 5 | 2 | 3 | 4 | 2 | 2 | 2 | 2.9 | 2 | 3 | 3 | 3 | 4 | 4 | . | . | . | 3.2 | 0 | 0 | 0 |
| ND071256 | Black | 3 | 3 | 3 | 3 | 3 | 2 | 3 | 3 | 4 | 3 | 2 | 1 | 1 | 2 | 2 | 2 | . | . | . | 1.7 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 0 | 0 | 0 |
| ND071257 | Black | 2 | 2 | 2 | 2 | 3 | 3 | 3 | 4 | . | 2.6 | 2 | 2 | 2 | 2 | 2 | 2 | . | . | . | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 0 | 0 | 0 |
| ND071281 | Black | 3 | 4 | 4 | 2 | 3 | 3 | 4 | 5 | 5 | 3.7 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 3 | 3 | 3 | . | . | . | 2.5 | 1 | 0 | 1 |
| ND071327 | Black | 2 | 3 | 3 | 2 | 3 | 3 | 3 | 3 | 3 | 2.8 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 5 | 5 | 5 | 5 | 5 | 5 | . | . | . | 5 | 1 | 0 | 1 |
| ND071333 | Black | 2 | 2 | 2 | 2 | 2 | 2 | . | . | . | 2 | 6 | 6 | 6 | 4 | 4 | 4 | 3 | 3 | 3 | 4.3 | 2 | 2 | 2 | 2 | 2 | 2 | 3 | 3 | 3 | 2.3 | 1 | 0 | 1 |
| ND071912 | Black | 2 | 2 | 2 | 5 | 5 | 5 | 4 | 4 | 4 | 3.7 | 2 | 2 | 3 | 2 | 3 | 4 | 6 | 7 | 7 | 4 | 3 | 3 | 3 | 3 | 3 | 3 | 4 | 4 | 4 | 3.3 | 1 | 0 | 1 |


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| ID code | Class | 1st trial |  |  |  |  |  |  |  |  |  | 2nd trial |  |  |  |  |  |  |  |  |  | 3rd trial |  |  |  |  |  |  |  |  |  | Marker data |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | Plant 1 |  |  | Plant 2 |  |  | Plant 3 |  |  | Mean | Plant 1 |  |  | Plant 2 |  |  | Plant 3 |  |  | Mean | Plant 1 |  |  | Plant 2 |  |  | Plant 3 |  |  | Mean |  |  |  |
|  |  | L1 | L2 | L3 | L1 | L2 | L3 | L1 | L2 | L3 |  | L1 | L2 | L3 | L1 | L2 | L3 | L1 | L2 | L3 |  | L1 | L2 | L3 | L1 | L2 | L3 | L1 | L2 | L3 |  | SAP6 | SU91 | Both |
| ND080284 | Pinto | 2 | 2 | 2 | 2 | 2 | 3 | . | . | . | 2.2 | 2 | 2 | 3 | 2 | 2 | 2 | 2 | 2 | 3 | 2.2 | 3 | 3 | 3 | 2 | 2 | 2 | . | . | . | 2.5 | 0 | 0 | 0 |
| ND080303 | Pinto | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 3 | . | . | . | 2.2 | . | . | . | . | . | . | . | . | . | . | 1 | 0 | 1 |
| ND080307 | Pinto | 2 | 2 | 2 | 3 | 2 | 2 | . | . | . | 2.2 | 2 | 2 | 2 | 2 | 2 | 2 | . | . | . | 2 | . | . | . | . | . | . | . | . | . | . | 1 | 0 | 1 |
| ND080313 | Pinto | 2 | 2 | 2 | 2 | 2 | 2 | . | . | . | 2 | 2 | 2 | 2 | 2 | 2 | 2 | . | . | . | 2 | 2 | 2 | 3 | 2 | 2 | 2 | . | . | . | 2.2 | 1 | 2 | 3 |
| ND080319 | Pinto | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 2 | 2 | 2 | 2 | 3 | . | . | . | 2.2 | 2 | 2 | 2 | 2 | 2 | 2 | 3 | 3 | 3 | 2.3 | 0 | 0 | 0 |
| ND080321 | Pinto | 3 | 4 | 4 | . | . | . | . | . | . | 3.7 | 2 | 3 | 3 | 3 | 3 | 4 | 4 | 5 | 5 | 3.6 | 3 | 3 | 3 | 3 | 3 | 2 | . | . | . | 2.8 | 1 | 0 | 1 |
| ND101301 | Pinto | 2 | 2 | 3 | 3 | 3 | 3 | 2 | 2 | 2 | 2.4 | 3 | 3 | 3 | . | . | . | . | . | . | 3 | 5 | 5 | 5 | 5 | 5 | 6 | 5 | 6 | 6 | 5.3 | 0 | 0 | 0 |
| ND101321 | Pinto | 3 | 3 | 3 | 2 | 2 | 2 | 3 | 3 | 3 | 2.7 | 3 | 3 | . | 3 | 3 | 2 | . | . | . | 2.8 | 2 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 2.9 | 1 | 0 | 1 |
| ND101322 | Pinto | 2 | 2 | 3 | 3 | 3 | 4 | . | . | . | 2.8 | 3 | 3 | 6 | 5 | 6 | 6 | . | . | . | 4.8 | 3 | 3 | 5 | 3 | 3 | 6 | 3 | 3 | 4 | 3.7 | 1 | 0 | 1 |
| ND101328 | Pinto | 3 | 3 | 3 | 2 | 3 | 3 | 2 | 3 | 3 | 2.8 | 4 | 4 | 5 | 2 | 2 | 2 | 2 | 2 | 3 | 2.9 | 3 | 3 | 3 | 5 | 5 | 5 | 3 | 3 | 3 | 3.7 | 0 | 0 | 0 |
| ND101330 | Pinto | 2 | 3 | 3 | 2 | 3 | 3 | 2 | 3 | 3 | 2.7 | 2 | 2 | 2 | 2 | 2 | 3 | 2 | 2 | 2 | 2.1 | 3 | 3 | 4 | . | . | . | . | . | . | 3.3 | 0 | 2 | 2 |
| ND101340 | Pinto | 3 | 4 | 5 | 5 | 5 | 5 |  | . | . | 4.5 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 3 | 3 | 2.2 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 0 | 0 | 0 |
| ND101341 | Pinto | 3 | 3 | 3 | 2 | 2 | 3 | 2 | 3 | 3 | 2.7 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 3 | 2.1 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 0 | 2 | 2 |
| ND101344 | Pinto | 2 | 2 | 4 | 2 | 2 | 2 | . | . | . | 2.3 | 2 | 2 | 3 | 3 | 3 | 3 | 2 | 3 | 3 | 2.7 | 2 | 2 | 2 | 3 | 3 | 3 | 3 | 3 | 3 | 2.7 | 0 | 2 | 2 |
| ND101350 | Pinto | 2 | 2 | 3 | 2 | 3 | 3 | 3 | 4 | 4 | 2.9 | 2 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 2.9 | 3 | 3 | 4 | 3 | 3 | 3 | . | . | . | 3.2 | 0 | 0 | 0 |
| ND101352 | Pinto | 2 | 3 | 3 | 2 | 3 | 3 | . | . | . | 2.7 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 3 | 3 | 2.2 | 2 | 2 | 2 | 2 | 2 | 3 | 2 | 2 | 4 | 2.3 | 0 | 0 | 0 |
| ND101353 | Pinto | 3 | 3 | 5 | 2 | 3 | 3 | 3 | 4 | 4 | 3.3 | 2 | 2 | 2 | . | . | . | . | . | . | 2 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 0 | 2 | 2 |
| ND101355 | Pinto | 3 | 3 | 4 | 3 | 3 | 3 | 2 | 2 | 3 | 2.9 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 2 | 2 | 3 | 3 | 3 | 3 | 3 | 3 | 2.7 | 0 | 0 | 0 |
| ND101358 | Pinto | 2 | 2 | 2 | 2 | 2 | 2 | . | . | . | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 3 | 3 | 2.2 | 1 | 0 | 1 |
| ND101371 | Pinto | 2 | 2 | 4 | 2 | 2 | 2 | 2 | 2 | 3 | 2.3 | 2 | 3 | 4 | 2 | 2 | 3 | . | . | . | 2.7 | 2 | 2 | 2 | 2 | 2 | 3 | 3 | 3 | 3 | 2.4 | 0 | 0 | 0 |
| ND121214 | Pinto | 2 | 3 | 3 | 2 | 2 | 2 | 2 | 2 | 2 | 2.2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 3 | 2 | 2.1 | 3 | 3 | 3 | 3 | 3 | 3 | . | . | . | 3 | 1 | 0 | 1 |
| ND121215 | Pinto | 2 | 3 | 3 | 3 | 3 | 4 | . | . | . | 3 | 2 | 2 | 2 | 2 | 2 | 2 | 3 | 3 | 5 | 2.6 | 2 | 2 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 2.8 | 1 | 0 | 1 |
| ND121217 | Pinto | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 2 | 2 | 2.7 | 4 | 4 | 4 | . | . | - | $\cdot$ | . | . | 4 | 2 | 2 | 3 | 2 | 3 | 3 | 3 | 3 | 3 | 2.7 | 0 | 0 | 0 |

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| ID code | Class | 1st trial |  |  |  |  |  |  |  |  |  | 2nd trial |  |  |  |  |  |  |  |  |  | 3rd trial |  |  |  |  |  |  |  |  |  | Marker data |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | Plant 1 |  |  | Plant 2 |  |  | Plant 3 |  |  | Mean | Plant 1 |  |  | Plant 2 |  |  | Plant 3 |  |  | Mean | Plant 1 |  |  | Plant 2 |  |  | Plant 3 |  |  | Mean |  |  |  |
|  |  | L1 | L2 | L3 | L1 | L2 | L3 | L1 | L2 | L3 |  | L1 | L2 | L3 | L1 | L2 | L3 | L1 | L2 | L3 |  | L1 | L2 | L3 | L1 | L2 | L3 | L1 | L2 | L3 |  | SAP6 | SU91 | Both |
| ND121229 | Pinto | 4 | 4 | 5 | 4 | 4 | 5 | 4 | 4 | 4 | 4.2 | 2 | 3 | 3 | 2 | 2 | 3 | 2 | 2 | 2 | 2.3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 0 | 2 | 2 |
| ND121230 | Pinto | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 5 | 5 | 5 | 4 | 4 | 6 | . | . | . | 4.8 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 0 | 0 | 0 |
| ND121231 | Pinto | 3 | 3 | 5 | 2 | 2 | 2 | 2 | 2 | 3 | 2.7 | 2 | 2 | 2 | 2 | 3 | 3 | . | . | . | 2.3 | 2 | 2 | 2 | 3 | 3 | 3 | . | . | . | 2.5 | 0 | 0 | 0 |
| ND121236 | Pinto | 2 | 2 | 3 | 2 | 3 | 3 | 2 | 3 | 4 | 2.7 | 2 | 2 | 2 | . | . | . | . | . | . | 2 | 6 | 5 | 5 | . | . | . | . | . | . | 5.3 | 1 | 0 | 1 |
| ND121237 | Pinto | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 3 | 2 | 2 | 2 | . | . | . | 2.2 | 3 | 3 | 2 | 3 | 3 | 3 | 3 | 3 | 3 | 2.9 | 1 | 2 | 3 |
| ND121241 | Pinto | 2 | 1 | 1 | 2 | 2 | 2 | 2 | 2 | 1 | 1.7 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 4 | 4 | 3.2 | 3 | 3 | 3 | 4 | 4 | 4 | . | . | . | 3.5 | 1 | 0 | 1 |
| ND121248 | Pinto | 3 | 3 | 3 | 2 | 3 | 3 | 2 | 3 | 3 | 2.8 | 2 | 2 | 3 | 3 | 3 | 4 | . | . | . | 2.8 | 2 | 3 | 3 | 2 | 2 | 2 | . | . | . | 2.3 | 1 | 2 | 3 |
| ND121260 | Pinto | 1 | 1 | . | 2 | 2 | 2 | 2 | 2 | 2 | 1.8 | 3 | 3 | 3 | 5 | 5 | 5 | . | . | . | 4 | 3 | 3 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 3.7 | 1 | 2 | 3 |
| ND121261 | Pinto | 5 | 6 | 6 | . | . | . | . | . | . | 5.7 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 3 | 2.1 | 3 | 3 | 2 | 2 | 2 | 2 | . | . | . | 2.3 | 1 | 0 | 1 |
| ND121265 | Pinto | 2 | 2 | 2 | 3 | 3 | 3 | . | . | . | 2.5 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 3 | 2.1 | 3 | 3 | 3 | . | . | . | . | . | . | 3 | 0 | 2 | 2 |
| ND121267 | Pinto | 3 | 3 | 4 | 3 | 3 | 3 | 5 | 5 | . | 3.6 | 3 | 3 | 4 | 4 | 4 | 5 | 4 | 4 | 5 | 4 | 3 | 3 | 3 | 4 | 4 | 4 | 4 | 4 | 3 | 3.6 | 0 | 0 | 0 |
| ND121268 | Pinto | 2 | 2 | 2 | 2 | 3 | 5 | 3 | 4 | 4 | 3 | 3 | 4 | 5 | 5 | 6 | 6 | 2 | 3 | 3 | 4.1 | 3 | 3 | 5 | 6 | 6 | 6 | 6 | 6 | 6 | 5.2 | 1 | 0 | 1 |
| ND121276 | Pinto | 2 | 2 | 3 | 2 | 3 | 3 | . | . | . | 2.5 | 2 | 2 | 2 | 2 | 3 | 3 | 2 | 2 | 2 | 2.2 | 2 | 2 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 2.8 | 0 | 0 | 0 |
| ND121277 | Pinto | 2 | 3 | 3 | 3 | 3 | 3 | 2 | 3 | 3 | 2.8 | 2 | 2 | 2 | 2 | 2 | 2 | . | . | . | 2 | 2 | 3 | 3 | 2 | 3 | 3 | . | . | . | 2.7 | 0 | 0 | 0 |
| ND121279 | Pinto | 4 | 5 | 5 | 4 | 4 | 5 | 4 | 4 | 4 | 4.3 | 2 | 2 | 2 | 2 | 3 | 3 | 3 | 3 | 3 | 2.6 | 3 | 3 | 2 | 3 | 2 | 2 | . | . | . | 2.5 | 0 | 0 | 0 |
| ND121281 | Pinto | 4 | 5 | 6 | 3 | 4 | 5 | 3 | 4 | 5 | 4.3 | 3 | 4 | 5 | 3 | 4 | 4 | 3 | 4 | 5 | 3.9 | 4 | 4 | 4 | 3 | 3 | 4 | 3 | 3 | 4 | 3.6 | 0 | 0 | 0 |
| ND121282 | Pinto | 4 | 4 | 5 | 3 | 3 | 3 | 3 | 4 | 4 | 3.7 | 4 | 4 | 5 | 2 | 2 | 7 | 2 | 3 | 3 | 3.6 | 2 | 2 | 3 | 3 | 3 | 4 | 3 | 3 | 4 | 3 | 0 | 2 | 2 |
| ND121283 | Pinto | 3 | 3 | 4 | 3 | 4 | 4 | . | . | . | 3.5 | 2 | 3 | 6 | 2 | 4 | 5 | 6 | 6 | 6 | 4.4 | 3 | 3 | 3 | 3 | 3 | 3 | . | . | . | 3 | 0 | 0 | 0 |
| ND121284 | Pinto | 5 | 5 | 5 | 2 | 3 | 3 | . | . | . | 3.8 | 3 | 3 | 3 | 4 | 4 | 6 | 4 | 4 | 4 | 3.9 | 3 | 6 | 6 | 3 | 3 | 3 | 4 | 4 | 4 | 4 | 0 | 2 | 2 |
| ND121285 | Pinto | 5 | 4 | 3 | 7 | 5 | 7 | 4 | 4 | 2 | 4.6 | 2 | 2 | 3 | 2 | 3 | 3 | 3 | 5 | 5 | 3.1 | 4 | 4 | 4 | 4 | 4 | 3 | 4 | 4 | 4 | 3.9 | 0 | 0 | 0 |
| ND121286 | Pinto | 5 | 5 | 5 | 5 | 5 | 4 | 4 | 5 | 5 | 4.8 | 2 | 3 | 3 | 2 | 3 | 3 | 3 | 3 | 3 | 2.8 | 2 | 2 | 2 | 3 | 3 | 3 | . | . | . | 2.5 | 0 | 2 | 2 |
| ND121290 | Pinto | 4 | 5 | 5 | 4 | 4 | 4 | 5 | 5 | 5 | 4.6 | 2 | 2 | 2 | 2 | 2 | 3 | . | . | . | 2.2 | . | . | . | . | . | . | . | . | . | . | 0 | 0 | 0 |
| ND121296 | Pinto | 4 | 4 | 6 | 5 | 6 | 6 | . | . | . | 5.2 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | . | . | . | . | . | . | 3 | 0 | 0 | 0 |







| ID code | Class | 1st trial |  |  |  |  |  |  |  |  |  | 2nd trial |  |  |  |  |  |  |  |  |  | 3rd trial |  |  |  |  |  |  |  |  |  | Marker data |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | Plant 1 |  |  | Plant 2 |  |  | Plant 3 |  |  | Mean | Plant 1 |  |  | Plant 2 |  |  | Plant 3 |  |  | Mean | Plant 1 |  |  | Plant 2 |  |  | Plant 3 |  |  | Mean |  |  |  |
|  |  | L1 | L2 | L3 | L1 | L2 | L3 | L1 | L2 | L3 |  | L1 | L2 | L3 | L1 | L2 | L3 | L1 | L2 | L3 |  | L1 | L2 | L3 | L1 | L2 | L3 | L1 | L2 | L3 |  | SAP6 | SU91 | Both |
| ND121580 | Pinto | 3 | 3 | 3 | 3 | 4 | 4 | 3 | 3 | 5 | 3.4 | 2 | 5 | 6 | . | . | . | . | . | . | 4.3 | 4 | 4 | 4 | . | . | . | . | . | . | 4 | 0 | 2 | 2 |
| ND121582 | Pinto | 4 | 4 | 4 | 4 | 4 | 4 | 3 | 4 | . | 3.9 | 2 | 2 | 4 | 2 | 2 | 2 | 2 | 2 | 2 | 2.2 | 2 | 2 | 2 | 2 | 2 | 2 | 3 | 3 | 3 | 2.3 | 1 | 0 | 1 |
| ND121606 | GN | 2 | 2 | 2 | 2 | 2 | 3 | 3 | 3 | 4 | 2.6 | 2 | 2 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 2.8 | 2 | 2 | 3 | 2 | 2 | 3 | 2 | 2 | 2 | 2.2 | 1 | 0 | 1 |
| ND121607 | GN | 2 | 2 | 2 | 2 | 2 | 4 | 2 | 2 | 4 | 2.4 | 2 | 2 | 2 | 3 | 3 | 3 | . | . | . | 2.5 | 3 | 3 | 3 | 3 | 3 | 3 | . | . | . | 3 | 1 | 0 | 1 |
| ND121610 | GN | 4 | 4 | 4 | 5 | 5 | 6 | 4 | 4 | 4 | 4.4 | 4 | 6 | . | 3 | 3 | 3 | . | . | . | 3.8 | 2 | 3 | 3 | 4 | 3 | 3 | 4 | 6 | 6 | 3.8 | 1 | 0 | 1 |
| ND121611 | GN | 3 | 3 | 3 | . | . | . | . | . | . | 3 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 0 | 0 | 0 |
| ND121612 | GN | 4 | 4 | 4 | 3 | 3 | 3 | 2 | 3 | 3 | 3.2 | 2 | 2 | 3 | 2 | 2 | 2 | 2 | 3 | 3 | 2.3 | 2 | 2 | 2 | 3 | 3 | 3 | 3 | 3 | 3 | 2.7 | 0 | 0 | 0 |
| ND121613 | GN | 3 | 3 | 4 | 2 | 2 | 3 | 3 | 4 | 4 | 3.1 | 3 | 3 | 4 | . | . | . | . | . | . | 3.3 | . | . | . | . | . | . | . | . | . | . | 0 | 0 | 0 |
| ND121614 | GN | 2 | 2 | 3 | 2 | 3 | 3 | 3 | 3 | 3 | 2.7 | 2 | 2 | 6 | 3 | 3 | 3 | 3 | 3 | 3 | 3.1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 0 | 0 | 0 |
| ND121615 | GN | 3 | 5 | 5 | 4 | 4 | 5 | 3 | 3 | 5 | 4.1 | 3 | 3 | 3 | 6 | 6 | 6 | . | . | . | 4.5 | 3 | 3 | 5 | 5 | 6 | 6 | . | . | . | 4.7 | 1 | 0 | 1 |
| ND121618 | GN | 3 | 2 | 2 | 3 | 3 | 3 | 3 | 3 | . | 2.8 | 2 | 2 | 2 | 2 | 2 | 2 | . | . | . | 2 | 3 | 3 | 3 | 4 | 4 | 4 | 3 | 3 | 3 | 3.3 | 1 | 0 | 1 |
| ND121619 | GN | 2 | 3 | 3 | 2 | 2 | 2 | 2 | 3 | 3 | 2.4 | 2 | 2 | 2 | 2 | 3 | 3 | 2 | 2 | 2 | 2.2 | 2 | 2 | 2 | 2 | 2 | 2 | . | . | . | 2 | 0 | 0 | 0 |
| ND121622 | GN | 2 | 2 | 2 | 4 | 5 | 5 | 4 | 6 | 7 | 4.1 | 2 | 3 | 3 | . | . | . | . | . | . | 2.7 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 1 | 0 | 1 |
| ND121625 | GN | 4 | 4 | 3 | 3 | 3 | 4 | 4 | 4 | 4 | 3.7 | 2 | 4 | 5 | 5 | 5 | 5 | 5 | 4 | 5 | 4.4 | 2 | 2 | 2 | 2 | 2 | 2 | . | . | . | 2 | 1 | 0 | 1 |
| ND121627 | GN | 3 | 3 | 4 | 5 | 5 | 5 | 3 | 5 | 5 | 4.2 | 3 | 4 | 4 | 3 | 3 | 4 | 6 | 6 | 6 | 4.3 | 5 | 5 | 5 | 3 | 4 | 4 | 4 | 3 | 4 | 4.1 | 1 | 0 | 1 |
| ND121630 | GN | 4 | 5 | 5 | 3 | 5 | 5 | 4 | 5 | 5 | 4.6 | 2 | 2 | 2 | 2 | 2 | 2 | . | . | . | 2 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 0 | 0 | 0 |
| ND121636 | GN | 4 | 4 | 7 | 5 | 5 | 5 | 5 | 5 | 6 | 5.1 | 2 | 4 | 4 | 2 | 2 | 2 | 3 | 3 | 3 | 2.8 | 3 | 3 | 3 | 3 | 3 | 3 | . | . | . | 3 | 1 | 0 | 1 |
| ND121637 | GN | 3 | 3 | 4 | 2 | 3 | 3 | 2 | 2 | 4 | 2.9 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | . | . | . | 2 | 1 | 0 | 1 |
| ND121640 | GN | 3 | 4 | 5 | 2 | 3 | 3 | . | . | . | 3.3 | 4 | 4 | 7 | . | . | . | . | . | . | 5 | 2 | 2 | 3 | 3 | 3 | 3 | . | . | . | 2.7 | 0 | 0 | 0 |
| ND121641 | GN | 2 | 2 | 2 | 3 | 3 | 5 | 3 | 4 | 4 | 3.1 | . | . | . | . | . | . | . | . | . | . | 2 | 2 | 2 | 3 | 3 | 3 | 3 | 3 | 3 | 2.7 | 0 | 0 | 0 |
| ND121642 | GN | 4 | 4 | 7 | 5 | 6 | 7 | . | . | . | 5.5 | 2 | 2 | 2 | 3 | 3 | 3 | 2 | 2 | 2 | 2.3 | 3 | 3 | 3 | 3 | 3 | 3 | . | . | . | 3 | 0 | 0 | 0 |
| ND121645 | GN | 4 | 5 | 5 | 3 | 3 | 4 | . | . | . | 4 | 4 | 6 | 7 | 2 | 2 | 3 | 4 | 7 | 7 | 4.7 | 3 | 3 | 3 | 4 | 4 | 5 | 3 | 3 | 3 | 3.4 | 0 | 0 | 0 |
| ND121647 | GN | 2 | 3 | 4 | 3 | 3 | 4 | 3 | 3 | 4 | 3.2 | 2 | 2 | 3 | 2 | 2 | 3 | 2 | 2 | 3 | 2.3 | 2 | 2 | 3 | 2 | 2 | 3 | $\cdot$ | . | . | 2.3 | 0 | 0 | 0 |


| ID code | Class | 1st trial |  |  |  |  |  |  |  |  |  | 2nd trial |  |  |  |  |  |  |  |  |  | 3rd trial |  |  |  |  |  |  |  |  |  | Marker data |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | Plant 1 |  |  | Plant 2 |  |  | Plant 3 |  |  | Mean | Plant 1 |  |  | Plant 2 |  |  | Plant 3 |  |  | Mean | Plant 1 |  |  | Plant 2 |  |  | Plant 3 |  |  | Mean |  |  |  |
|  |  | L1 | L2 | L3 | L1 | L2 | L3 | L1 | L2 | L3 |  | L1 | L2 | L3 | L1 | L2 | L3 | L1 | L2 | L3 |  | L1 | L2 | L3 | L1 | L2 | L3 | L1 | L2 | L3 |  | SAP6 | SU91 | Both |
| ND121648 | GN | 5 | 6 | 4 | . | . | . | . | . | . | 5 | 2 | 2 | 2 | 6 | 6 | 6 | . | . | . | 4 | 3 | 3 | 4 | 3 | 3 | 3 | . | . | . | 3.2 | 0 | 0 | 0 |
| ND121652 | GN | 2 | 4 | 4 | 3 | 3 | 3 | 3 | 4 | 4 | 3.3 | 2 | 2 | 3 | 3 | 3 | 5 | . | . | . | 3 | 3 | 3 | 3 | 3 | 3 | 3 | . | . | . | 3 | 1 | 0 | 1 |
| ND121656 | GN | 3 | 3 | . | 2 | 2 | 2 | 2 | 2 | 2 | 2.3 | 3 | 3 | 3 | 4 | 4 | 5 | . | . | . | 3.7 | 5 | 5 | 5 | 3 | 4 | 4 | . | . | . | 4.3 | 0 | 0 | 0 |
| ND121657 | GN | 4 | 4 | 6 | 4 | 5 | 6 | 2 | 2 | 2 | 3.9 | 3 | 3 | 9 | 4 | 3 | 3 | 3 | 3 | 4 | 3.9 | 6 | 6 | 6 | . | . | . | . | . | . | 6 | 0 | 0 | 0 |
| ND121666 | GN | 3 | 4 | 7 | 3 | 4 | 6 | 4 | 5 | 5 | 4.6 | 3 | 3 | 3 | 3 | 3 | 3 | . | . | . | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 4 | 3.1 | 0 | 0 | 0 |
| ND121667 | GN | 3 | 3 | 3 | 4 | 4 | 4 | 2 | 3 | 3 | 3.2 | 3 | 2 | 2 | 4 | 4 | 4 | . | . | . | 3.2 | 3 | 3 | 3 | 2 | 3 | 3 | . | . | . | 2.8 | 0 | 0 | 0 |
| ND121668 | GN | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 2.9 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 0 | 0 | 0 |
| ND121672 | GN | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 3 | 3 | 2 | 2 | 2 | 2 | 2 | 2 | 2.2 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 1 | 0 | 1 |
| ND121676 | GN | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 4 | 3 | 4 | 4 | . | . | . | 3.5 | 3 | 3 | 4 | 3 | 3 | 4 |  | . | . | 3.3 | 0 | 0 | 0 |
| ND121677 | GN | 3 | 3 | 3 | 3 | 4 | 4 | . | . | . | 3.3 | 2 | 2 | 2 | 2 | 2 | 3 | 3 | 3 | 3 | 2.4 | 2 | 2 | 3 | 2 | 3 | 3 | 3 | 3 | 2 | 2.6 | 1 | 0 | 1 |
| ND121679 | GN | 3 | 3 | 3 | 5 | 5 | 5 | 4 | 4 | 5 | 4.1 | 2 | 2 | 3 | 2 | 2 | 2 | 2 | 2 | 2 | 2.1 | 5 | 5 | 5 | 3 | 3 | 3 | 3 | 3 | 3 | 3.7 | 1 | 0 | 1 |
| ND121680 | GN | 2 | 2 | 2 | 2 | 2 | 2 | . | . | . | 2 | 2 | 2 | 2 | 2 | 3 | 4 | 2 | 3 | 2 | 2.4 | 2 | 2 | 3 | 2 | 2 | 2 | 2 | 2 | 2 | 2.1 | 1 | 0 | 1 |
| ND121681 | GN | 2 | 2 | 3 | 3 | 3 | 3 | . | . | . | 2.7 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 3 | 3 | 2 | 2 | 2 | 2 | 2 | 2 | 3 | 2.3 | 1 | 0 | 1 |
| ND121682 | GN | 3 | 3 | 3 | 3 | 3 | 3 | 4 | 4 | 4 | 3.3 | 2 | 2 | 3 | 3 | 5 | 6 | 3 | 3 | 3 | 3.3 | 3 | 4 | 5 | 4 | 4 | 4 | . | . | . | 4 | 1 | 0 | 1 |
| ND121686 | GN | 3 | 4 | 5 | 3 | 3 | 3 | . | . | . | 3.5 | 2 | 2 | 2 | 3 | 3 | 3 | 3 | 3 | 3 | 2.7 | 2 | 2 | 3 | 3 | 3 | 3 | . | . | . | 2.7 | 1 | 0 | 1 |
| ND121687 | GN | 2 | 2 | 2 | 3 | 3 | 3 | 3 | 3 | 3 | 2.7 | 2 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 4 | 3 | 2 | 3 | 3 | 3 | 3 | 4 | 3 | 3 | 4 | 3.1 | 0 | 0 | 0 |
| ND121689 | GN | 3 | 3 | 3 | 3 | 4 | . | 2 | 3 | 3 | 3 | 2 | 2 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 2.8 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 1 | 0 | 1 |
| ND121697 | GN | 3 | 4 | 4 | 4 | 7 | 7 | 2 | 2 | 2 | 3.9 | 3 | 4 | 4 | 4 | 4 | 4 | . | . | . | 3.8 | 6 | 6 | 6 | 5 | 6 | 6 | 5 | 5 | 6 | 5.7 | 0 | 0 | 0 |
| ND121699 | GN | 3 | 3 | 3 | . | . | . | . | . | . | 3 | 3 | 3 | 3 | 2 | 2 | 3 | 2 | 2 | 2 | 2.4 | 3 | 3 | 4 | 4 | 4 | 4 | 3 | 3 | 4 | 3.6 | 0 | 0 | 0 |
| ND121700 | GN | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 4 | 4 | 5 | 5 | 4 | 5 | 5 | 4 | 4 | 4.4 | 0 | 0 | 0 |
| ND121704 | GN | 3 | 3 | 3 | . | . | $\cdot$ | . | . | . | 3 | 2 | 2 | 2 | 2 | 2 | 2 | . | . | . | 2 | 2 | 3 | 3 | 3 | 3 | 3 | . | . | . | 2.8 | 0 | 0 | 0 |
| ND121705 | GN | 6 | 8 | 8 | 3 | 3 | 3 | . | . | . | 5.2 | 9 | 9 | 9 | 7 | 7 | 7 | . | . | . | 8 | 6 | 6 | 6 | . | . | . | . | . | . | 6 | 0 | 0 | 0 |
| ND121708 | GN | 2 | 3 | 3 | 2 | 2 | 2 | 2 | 2 | 2 | 2.2 | 2 | 2 | . | 2 | 2 | 2 | . | . | . | 2 | 2 | 2 | 3 | 2 | 2 | 3 | 2 | 2 | 2 | 2.2 | 0 | 0 | 0 |


| ID code | Class | 1st trial |  |  |  |  |  |  |  |  |  | 2nd trial |  |  |  |  |  |  |  |  |  | 3rd trial |  |  |  |  |  |  |  |  |  | Marker data |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | Plant 1 |  |  | Plant 2 |  |  | Plant 3 |  |  | Mean | Plant 1 |  |  | Plant 2 |  |  | Plant 3 |  |  | Mean | Plant 1 |  |  | Plant 2 |  |  | Plant 3 |  |  | Mean |  |  |  |
|  |  | L1 | L2 | L3 | L1 | L2 | L3 | L1 | L2 | L3 |  | L1 | L2 | L3 | L1 | L2 | L3 | L1 | L2 | L3 |  | L1 | L2 | L3 | L1 | L2 | L3 | L1 | L2 | L3 |  | SAP6 | SU91 | Both |
| ND121709 | GN | 2 | 2 | 2 | . | . | . | . | . | . | 2 | 8 | 8 | 8 | 9 | . | . | . | . | . | 8.3 | 7 | 7 | 8 | 8 | 8 | 8 | 7 | 7 | 7 | 7.4 | 0 | 0 | 0 |
| ND121710 | GN | 2 | 3 | 3 | 1 | 1 | 3 | . | . | . | 2.2 | 2 | 2 | 3 | 3 | 3 | 3 | . | . | . | 2.7 | 3 | 3 | 3 | 2 | 2 | 3 | . | . | . | 2.7 | 1 | 0 | 1 |
| ND121716 | GN | 3 | 4 | 4 | 2 | 2 | 2 | . | . | . | 2.8 | 3 | 3 | 3 | . | . | . | . | . | . | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 4 | 4 | 4 | 3.3 | 1 | 0 | 1 |
| ND121717 | GN | 2 | 3 | 4 | 6 | 6 | 8 | . | . | . | 4.8 | 3 | 3 | 3 | . | . | . | . | . | . | 3 | 3 | 3 | 3 | 2 | 3 | 3 | 3 | 3 | 3 | 2.9 | 1 | 0 | 1 |
| ND121718 | GN | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 3 | 3 | 3 | 2 | 2 | 2 | 2.3 | 1 | 0 | 1 |
| ND121719 | GN | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 3 | 2.1 | 2 | 3 | 3 | 2 | 2 | 2 | 2 | 2 | 2 | 2.2 | 4 | 4 | 5 | 4 | 5 | 5 | . | . | . | 4.5 | 1 | 0 | 1 |
| ND121721 | GN | 5 | 5 | 5 | 2 | 3 | 3 | 2 | 3 | 3 | 3.4 | 2 | 3 | 3 | 2 | 3 | 3 | . | . | . | 2.7 | 4 | 4 | 5 | 5 | 5 | 6 | 5 | 5 | 4 | 4.8 | 1 | 0 | 1 |
| ND121723 | GN | 3 | 3 | 3 | 6 | 6 | . | . | . | . | 4.2 | 2 | 3 | 3 | 3 | 3 | 5 | 2 | 3 | 3 | 3 | 4 | 4 | 4 | 3 | 3 | 4 | 4 | 4 | 4 | 3.8 | 1 | 0 | 1 |
| ND121725 | GN | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 3 | 3 | 2.9 | 2 | 2 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 2.8 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 1 | 0 | 1 |
| ND121727 | GN | 5 | 5 | 7 | 5 | 5 | 5 | . | . | . | 5.3 | 2 | 2 | 3 | 2 | 3 | 3 | . | . | . | 2.5 | 3 | 3 | 3 | 2 | 3 | 3 | . | . | . | 2.8 | 1 | 0 | 1 |
| ND121733 | GN | 2 | 3 | 3 | 4 | 3 | 3 | 4 | 8 | 8 | 4.2 | 3 | 3 | 4 | 3 | 5 | 4 | 2 | 4 | 4 | 3.6 | 3 | 3 | 4 | 3 | 3 | 3 | 3 | 3 | 4 | 3.2 | 1 | 0 | 1 |
| ND121735 | GN | 4 | 7 | 7 | 3 | 4 | 8 | 3 | 3 | 5 | 4.9 | 2 | 2 | 2 | 4 | 4 | 4 | . | . | . | 3 | 4 | 4 | 4 | 2 | 2 | 2 | 3 | 4 | 4 | 3.2 | 1 | 0 | 1 |
| ND121739 | GN | 2 | 2 | 4 | 1 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | . | . | . | 2 | 2 | 4 | 4 | 4 | 4 | 3 | 3 | 3 | 3 | 3.3 | 1 | 0 | 1 |
| ND121741 | GN | 2 | 2 | 3 | 3 | 3 | 3 | . | . | . | 2.7 | 2 | 2 | 2 | 2 | 2 | 3 | 2 | 2 | 2 | 2.1 | 4 | 4 | 5 | 5 | 5 | 5 | 4 | 4 | 4 | 4.4 | 1 | 0 | 1 |
| ND112811 | GN | 3 | 3 | 3 | 4 | 4 | 5 | 3 | 3 | . | 3.5 | 2 | 3 | 3 | 3 | 3 | 3 | . | . | . | 2.8 | 4 | 4 | 4 | 4 | 4 | 4 | . | . | . | 4 | 1 | 0 | 1 |
| ND112820 | GN | 2 | 3 | 3 | 2 | 3 | 3 | . | . | . | 2.7 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 3 | 2.1 | 2 | 2 | 3 | 2 | 3 | 3 |  | . | . | 2.5 | 0 | 0 | 0 |
| ND112831 | GN | 3 | 7 | 7 | . | . | . | . | . | . | 5.7 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 3 | 2.1 | 0 | 0 | 0 |
| ND112836 | GN | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 3 | 3 | 2.2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 4 | 4 | 3 | 3 | 3 | 4 | 3 | 4 | 4 | 3.6 | 0 | 0 | 0 |
| ND112838 | GN | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 3 | 2.1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 3 | 2 | 2 | 3 | 2.2 | 0 | 0 | 0 |
| MERLOT | SR | 2 | 3 | 3 | 5 | 5 | 5 | 3 | 4 | 4 | 3.8 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 1 | 0 | 1 |
| ND112839 | GN | 2 | 3 | 3 | 2 | 2 | 2 | 2 | 2 | . | 2.3 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 3 | 3 | 3 | 3 | 3 | 2 | 3 | 2 | 2.7 | 0 | 0 | 0 |
| ND112843 | GN | 4 | 4 | 4 | . | . | . | . | . | . | 4 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 3 | 3 | 3 | 2.3 | 0 | 0 | 0 |
| RIO ROJO | SR | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | . | . | . | . | . | . | . | . | . | . | 2 | 2 | 3 | 3 | 3 | 3 | 3 | 3 | 4 | 2.9 | 0 | 2 | 2 |


| ID code | Class | 1st trial |  |  |  |  |  |  |  |  |  | 2nd trial |  |  |  |  |  |  |  |  |  | 3rd trial |  |  |  |  |  |  |  |  |  | Marker data |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | Plant 1 |  |  | Plant 2 |  |  | Plant 3 |  |  | Mean | Plant 1 |  |  | Plant 2 |  |  | Plant 3 |  |  | Mean | Plant 1 |  |  | Plant 2 |  |  | Plant 3 |  |  | Mean |  |  |  |
|  |  | L1 | L2 | L3 | L1 | L2 | L3 | L1 | L2 | L3 |  | L1 | L2 | L3 | L1 | L2 | L3 | L1 | L2 | L3 |  | L1 | L2 | L3 | L1 | L2 | L3 | L1 | L2 | L3 |  | SAP6 | SU91 | Both |
| SEDONA | Pink | 2 | 2 | 2 | 3 | 3 | . | . | . | . | 2.4 | 2 | 3 | 4 | 2 | 2 | 2 | 3 | 3 | - | 2.6 | 3 | 3 | 3 | 4 | 4 | 3 | . | . | . | 3.3 | 1 | 0 | 1 |
| ND112901 | SR | 2 | 2 | 2 | 3 | 3 | 4 | 3 | 3 | 3 | 2.8 | 2 | 2 | 2 | 2 | 2 | 3 | . | . | . | 2.2 | 5 | 5 | 5 | 5 | 6 | 6 | 4 | 4 | 4 | 4.9 | 1 | 2 | 3 |
| ND112905 | Pink | 3 | 3 | 3 | 3 | 3 | 3 | . | . | . | 3 | 3 | 3 | 3 | 2 | 2 | 3 | 2 | 2 | 2 | 2.4 | 3 | 3 | 3 | 3 | 3 | 3 | . | . | . | 3 | 1 | 0 | 1 |
| ND112907 | Pink | 4 | 5 | 7 | 5 | 5 | 5 | 3 | 3 | 3 | 4.4 | 3 | . | . | 3 | 3 | 3 | 2 | 2 | 3 | 2.7 | 3 | 3 | 3 | 3 | 3 | 4 | 3 | 4 | 4 | 3.3 | 1 | 0 | 1 |
| ND112908 | SR | 4 | 4 | 4 | 2 | 3 | 3 | 2 | 2 | 4 | 3.1 | 4 | 4 | 5 | 3 | 4 | 5 | 2 | 3 | 3 | 3.7 | 3 | 4 | 5 | 3 | 3 | 3 | 3 | 3 | 4 | 3.4 | 1 | 0 | 1 |
| ND112914 | SR | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 2 | 3 | 3 | 3 | 3 | . | . | . | 2.7 | 2 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 2.9 | 1 | 0 | 1 |
| ND112915 | SR | 3 | 3 | 3 | 3 | 3 | . | 2 | 3 | . | 2.9 | 3 | 3 | 3 | . | . | . | . | . | . | 3 | 4 | 3 | 3 | 3 | 4 | 4 | 4 | 4 | 4 | 3.7 | 1 | 2 | 3 |
| ND112916 | SR | 2 | 2 | 3 | . | . | . | 2 | 2 | 2 | 2.2 | 3 | 3 | 6 | 3 | 3 | 3 | . | . | . | 3.5 | 2 | 2 | 3 | 4 | 4 | 4 | . | . | . | 3.2 | 1 | 2 | 3 |
| ND112918 | SR | 2 | 2 | 3 | 2 | 5 | 8 | . | . | . | 3.7 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | . | . | . | . | . | . | . | . | . | . | 1 | 2 | 3 |
| ND112927 | SR | 2 | 2 | 2 | 4 | 5 | 5 | 3 | 3 | 3 | 3.2 | 3 | 3 | 3 | 2 | 2 | 2 | 2 | 2 | 2 | 2.3 | 4 | 4 | 4 | 5 | 5 | 5 | . | . | . | 4.5 | 0 | 2 | 2 |
| ND112929 | Pink | 3 | 3 | 3 | 2 | 2 | 2 | 3 | 3 | 3 | 2.7 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 3 | 3 | 2 | 2 | 2 | 3 | 3 | 3 | 4 | 2.8 | 0 | 2 | 2 |
| ND112930 | Pink | 5 | 5 | 5 | 4 | 5 | . | . | . | . | 4.8 | 3 | 3 | 3 | 3 | 3 | 4 | 3 | 3 | 3 | 3.1 | 3 | 3 | 4 | 4 | 4 | 4 | 3 | 3 | 4 | 3.6 | 0 | 0 | 0 |
| ND112935 | Pink | 3 | 2 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 2.9 | 2 | 2 | 2 | 2 | 3 | 3 | . | . | . | 2.3 | 2 | 3 | 3 | 2 | 3 | 3 | 2 | 3 | 3 | 2.7 | 1 | 0 | 1 |
| ND112938 | SR | 3 | 3 | 3 | 3 | 3 | 3 | . | . | . | 3 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 3 | 3 | 3 | 3 | 2 | 2 | 2 | 2.4 | 0 | 2 | 2 |
| ND112947 | SR | 3 | 3 | 5 | 5 | 5 | 5 | . | . | . | 4.3 | 2 | 2 | 2 | 3 | 3 | 3 | . | . | . | 2.5 | 3 | 3 | 3 | 2 | 3 | 3 | 3 | 3 | 3 | 2.9 | 1 | 0 | 1 |
| ND112948 | SR | 3 | 4 | 5 | 2 | 2 | 2 | 2 | 2 | 2 | 2.7 | 2 | 5 | 5 | 5 | 5 | 5 | 2 | 4 | 5 | 4.2 | 4 | 4 | 4 | 5 | 5 | 5 | 4 | 4 | 3 | 4.2 | 1 | 0 | 1 |
| ND112949 | SR | 2 | 2 | 2 | 3 | 3 | 3 | . | . | . | 2.5 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 3 | 3 | 3 | 4 | 4 | 3 | 3 | 3 | 3 | 3.2 | 1 | 0 | 1 |
| ND112950 | SR | 3 | 3 | 3 | 3 | 3 | 3 | . | . | . | 3 | . | . | . | . | . | . | . | . | . | . | 3 | 3 | 4 | 4 | 4 | 5 | 6 | 6 | 6 | 4.6 | 1 | 0 | 1 |
| ND112951 | SR | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | . | 3 | 2 | 2 | 2 | 4 | 7 | . | 2 | 2 | 2 | 2.9 | 4 | 4 | 6 | 4 | 4 | 5 | . | . | . | 4.5 | 0 | 0 | 0 |
| ND112956 | Pink | 6 | 2 | 2 | 4 | 2 | 2 | 6 | 2 | 2 | 3.1 | 3 | 3 | 3 | 2 | 2 | 2 | 2 | 2 | 2 | 2.3 | 4 | 4 | 4 | 4 | 4 | 5 | 5 | 5 | 5 | 4.4 | 1 | 0 | 1 |
| ND112966 | Pink | 7 | 7 | 6 | 4 | 4 | 6 | 7 | 7 | 7 | 6.1 | 2 | 3 | 5 | 4 | 5 | 5 | . | . | . | 4 | 6 | 6 | 7 | 5 | 6 | 6 | . | . | . | 6 | 1 | 0 | 1 |
| ND112973 | Pink | 4 | 4 | 4 | 3 | 3 | 3 | . | . | . | 3.5 | 4 | 4 | 6 | 4 | 5 | 5 | 4 | 4 | 4 | 4.4 | 4 | 4 | 5 | 5 | 5 | 5 | . | . | . | 4.7 | 0 | 0 | 0 |
| ND112978 | SR | 4 | 4 | 4 | 6 | 5 | 9 | . | . | . | 5.3 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 2 | 3 |





| ID code | Class | 1st trial |  |  |  |  |  |  |  |  |  | 2nd trial |  |  |  |  |  |  |  |  |  | 3rd trial |  |  |  |  |  |  |  |  |  | Marker data |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | Plant 1 |  |  | Plant 2 |  |  | Plant 3 |  |  | Mean | Plant 1 |  |  | Plant 2 |  |  | Plant 3 |  |  | Mean | Plant 1 |  |  | Plant 2 |  |  | Plant 3 |  |  | Mean |  |  |  |
|  |  | L1 | L2 | L3 | L1 | L2 | L3 | L1 | L2 | L3 |  | L1 | L2 | L3 | L1 | L2 | L3 | L1 | L2 | L3 |  | L1 | L2 | L3 | L1 | L2 | L3 | L1 | L2 | L3 |  | SAP6 | SU91 | Both |
| ND122095 | Navy | 3 | 6 | 9 | 9 | 9 | 9 | 4 | 5 | 3 | 6.3 | 4 | 4 | 4 | 6 | 6 | 6 | . | . | . | 5 | 5 | 5 | 6 | 6 | 6 | 6 | 5 | 5 | 5 | 5.4 | 1 | 0 | 1 |
| ND122098 | Navy | 3 | 3 | 8 | 5 | 4 | 4 | 5 | 7 | 9 | 5.3 | 6 | 6 | 6 | 6 | 7 | 7 | 6 | 6 | 7 | 6.3 | 4 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 5.8 | 1 | 0 | 1 |
| ND122099 | Navy | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 7 | 9 | 9 | 9 | 9 | 9 | 8.8 | 7 | 7 | 7 | 8 | 8 | 8 | 7 | 7 | 9 | 7.6 | 1 | 0 | 1 |
| ND122100 | Navy | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 5 | 7 | 7 | 6 | 7 | 7 | . | . | . | 6.5 | 6 | 7 | 7 | 7 | 7 | 7 | . | . | . | 6.8 | 1 | 0 | 1 |
| ND122109 | Navy | 9 | 9 | 9 | 9 | 9 | 8 | 7 | 7 | 7 | 8.2 | 7 | 7 | 9 | . | . | . | . | . | . | 7.7 | 6 | 6 | 7 | 6 | 6 | 7 | . | . | . | 6.3 | 1 | 0 | 1 |
| ND122110 | Navy | 3 | 3 | 3 | 4 | 4 | 4 | 5 | 5 | 5 | 4 | 4 | 5 | 6 | 5 | 5 | 5 | 6 | 6 | 6 | 5.3 | 6 | 6 | 6 | 6 | 6 | 5 | 6 | 6 | 5 | 5.8 | 1 | 0 | 1 |
| ND122114 | Navy | 5 | 5 | 8 | 3 | 3 | 3 | 3 | 3 | 3 | 4 | 3 | 3 | 3 | 4 | 5 | 5 | . | . | . | 3.8 | 3 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 5 | 3.9 | 1 | 0 | 1 |
| ND122115 | Navy | 9 | 9 | 9 | . | . | . | . | . | . | 9 | 5 | 6 | 6 | 7 | 7 | 7 | 9 | 9 | 9 | 7.2 | 7 | 7 | 7 | 7 | 9 | 9 | 9 | 9 | 9 | 8.1 | 1 | 0 | 1 |
| ND122116 | Navy | 4 | 4 | 4 | 3 | 3 | 3 | 4 | 4 | 4 | 3.7 | 4 | 4 | 4 | 4 | 7 | 7 | 7 | 7 | 7 | 5.7 | 4 | 4 | 4 | 5 | 5 | 5 | 4 | 5 | 5 | 4.6 | 1 | 0 | 1 |
| ND122118 | Navy | 9 | 9 | 8 | 9 | 9 | 9 | 9 | 9 | 9 | 8.9 | 9 | 9 | 9 | 7 | 7 | 9 | 6 | 7 | 7 | 7.8 | 6 | 6 | 6 | 5 | 5 | 5 | 7 | 7 | 7 | 6 | 1 | 0 | 1 |
| ND122124 | Navy | . | . | . | . | . | . | . | . | . | . | 4 | 4 | 4 | 4 | 6 | 6 | . | . | . | 4.7 | 5 | 5 | 6 | 5 | 5 | 5 | 6 | 6 | 6 | 5.4 | 1 | 0 | 1 |
| ND122125 | Navy | 3 | 3 | 3 | 3 | 3 | 4 | 3 | 3 | 3 | 3.1 | . | . | . | 4 | 6 | 6 | 4 | 5 | 2 | 4.5 | 3 | 3 | 3 | 4 | 4 | 4 |  | . | . | 3.5 | 1 | 0 | 1 |
| ND122130 | Navy | 9 | 9 | 9 | 3 | 4 | 7 | 7 | 7 | 7 | 6.9 | 4 | 6 | 6 | 4 | 7 | 6 | 6 | 6 | 7 | 5.8 | 6 | 6 | 6 | 5 | 5 | 6 | . | . | . | 5.7 | 1 | 0 | 1 |
| ND122137 | Navy | 6 | 6 | 6 | 6 | 6 | 7 | x | x | x | 6.2 | 5 | 5 | 6 | . | . | . | . | . | . | 5.3 | 6 | 6 | 6 | 6 | 6 | 7 | 5 | 6 | 6 | 6 | 1 | 0 | 1 |
| ND122143 | Navy | 3 | 3 | 4 | 3 | 4 | 5 | 3 | 3 | 3 | 3.4 | 3 | 3 | 3 | 3 | 3 | 3 | . | . | . | 3 | 5 | 5 | 5 | 4 | 4 | 3 | 3 | 3 | 3 | 3.9 | 1 | 0 | 1 |
| ND122145 | Navy | 3 | 3 | 3 | 4 | 5 | 6 | 4 | 7 | 9 | 4.9 | 6 | 6 | 6 | 6 | 7 | 7 | 4 | 4 | 4 | 5.6 | 6 | 6 | 6 | 7 | 7 | 7 | 6 | 7 | 7 | 6.6 | 1 | 0 | 1 |
| ND122146 | Navy | 5 | 5 | 6 | . | . | . | . | . | . | 5.3 | 4 | 5 | 5 | 9 | 9 | 9 | . | . | . | 6.8 | 6 | 7 | 7 | 7 | 7 | 8 | 7 | 7 | 7 | 7 | 1 | 0 | 1 |
| ND122150 | Navy | 3 | 3 | 3 | 3 | 3 | 4 | 3 | 3 | 3 | 3.1 | . | . | . | . | . | . | . | . | . | . | 3 | 3 | 3 | 3 | 3 | 4 | . | . | . | 3.2 | 1 | 0 | 1 |
| ND122151 | Navy | 2 | 2 | 2 | 3 | 3 | 3 | 3 | 3 | 3 | 2.7 | 3 | 3 | 4 | . | . | . | . | . | . | 3.3 | 3 | 3 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 3.7 | 1 | 0 | 1 |
| ND122152 | Navy | 3 | 3 | 4 | 3 | 7 | 7 | . | . | . | 4.5 | 5 | 5 | 6 | 5 | 6 | 6 | . | . | . | 5.5 | 6 | 6 | 6 | 6 | 6 | 5 | . | . | . | 5.8 | 1 | 0 | 1 |
| ND122155 | Navy | 6 | 5 | 8 | 5 | 5 | . | . | . | . | 5.8 | 6 | 6 | 6 | 4 | 6 | 6 | . | . | . | 5.7 | 6 | 7 | 7 | 6 | 6 | 6 | 6 | 7 | 7 | 6.4 | 1 | 0 | 1 |
| ND122159 | Navy | 7 | 7 | 7 | 3 | 7 | 7 | . | . | . | 6.3 | 4 | 4 | 4 | 4 | 4 | 4 | . | . | . | 4 | 4 | 4 | 5 | 4 | 4 | 4 | 4 | 4 | 5 | 4.2 | 1 | 0 | 1 |
| ND122163 | Navy | e | e | e | 4 | 6 | 8 | 6 | 6 | 8 | 6.3 | 6 | 6 | 8 | 8 | 8 | 8 | . | $\cdot$ | . | 7.3 | 6 | 6 | 6 | 5 | 6 | 6 | 5 | 6 | 6 | 5.8 | 1 | 0 | 1 |



| ID code | Class | 1st trial |  |  |  |  |  |  |  |  |  | 2nd trial |  |  |  |  |  |  |  |  |  | 3rd trial |  |  |  |  |  |  |  |  |  | Marker data |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | Plant 1 |  |  | Plant 2 |  |  | Plant 3 |  |  | Mean | Plant 1 |  |  | Plant 2 |  |  | Plant 3 |  |  | Mean | Plant 1 |  |  | Plant 2 |  |  | Plant 3 |  |  | Mean |  |  |  |
|  |  | L1 | L2 | L3 | L1 | L2 | L3 | L1 | L2 | L3 |  | L1 | L2 | L3 | L1 | L2 | L3 | L1 | L2 | L3 |  | L1 | L2 | L3 | L1 | L2 | L3 | L1 | L2 | L3 |  | SAP6 | SU91 | Both |
| NDF120107 | Black | 3 | 4 | 6 | 4 | 4 | . | . | . | . | 4.2 | 2 | 2 | 3 | 2 | 2 | 2 | 2 | 2 | 2 | 2.1 | 3 | 3 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 3.7 | 1 | 0 | 1 |
| NDF120108 | Black | 3 | 3 | 5 | 3 | 3 | 4 | 3 | 3 | 3 | 3.3 | 3 | 3 | 3 | 3 | 3 | 4 | 4 | 4 | 4 | 3.4 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 1 | 0 | 1 |
| NDF120114 | Black | 3 | 3 | 3 | 6 | 6 | 5 | 6 | 6 | 6 | 4.9 | 3 | 3 | 4 | 6 | 7 | 7 | 3 | 3 | 5 | 4.6 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 0 | 0 | 0 |
| NDF120116 | Black | 3 | 3 | 4 | 3 | 3 | 3 | 5 | 6 | 9 | 4.3 | 4 | 4 | 5 | 4 | 4 | 4 | 4 | 5 | 5 | 4.3 | 3 | 3 | 3 | 4 | 4 | 4 | 3 | 3 | 3 | 3.3 | 1 | 0 | 1 |
| NDF120121 | Black | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 5 | 5 | 6 | 3 | 3 | 3 | 3 | 3 | 3 | 3.8 | 2 | 2 | 2 | 3 | 3 | 3 | 3 | 3 | 3 | 2.7 | 1 | 0 | 1 |
| NDF120128 | Black | . | . | . | 2 | 3 | 3 | 2 | 3 | 4 | 2.8 | 3 | 4 | 4 | 2 | 2 | 3 | . | . | . | 3 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 0 | 1 |
| NDF120133 | Black | 3 | 3 | 4 | 6 | 6 | 7 | . | . | . | 4.8 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 3 | 2.1 | 1 | 0 | 1 |
| NDF120135 | Black | 3 | 3 | 9 | 3 | 3 | 5 | 3 | 3 | 3 | 3.9 | 3 | 5 | 6 | 5 | 5 | 5 | 7 | 7 | 7 | 5.6 | 3 | 3 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 3.7 | 1 | 0 | 1 |
| NDF120141 | Black | 6 | 7 | 8 | 3 | 3 | 3 | 3 | 3 | 3 | 4.3 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 1 | 0 | 1 |
| NDF120157 | Black | 3 | 4 | 6 | 3 | 3 | 5 | 2 | 3 | 7 | 4 | 3 | 3 | 4 | 3 | 3 | 3 | 4 | 4 | 4 | 3.4 | 3 | 3 | 3 | 4 | 4 | 4 | 3 | 3 | 3 | 3.3 | 0 | 0 | 0 |
| NDF120164 | Black | . | . | . | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 2 | 2 | 2 | 2 | 2 | . | . | . | 2 | 3 | 3 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 3.7 | 0 | 0 | 0 |
| NDF120165 | Black | 3 | 3 | 3 | 3 | 3 | 3 | . | . | . | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 4 | 4 | 4 | 6 | 6 | 6 | 5 | 5 | 5 | 5 | 1 | 0 | 1 |
| NDF120168 | Black | 8 | 7 | 4 | 3 | 3 | 9 | 3 | 3 | 3 | 4.8 | 4 | 4 | 5 | 3 | 3 | 3 | 3 | 3 | 3 | 3.4 | 2 | 2 | 2 | 3 | 3 | 3 | 2 | 2 | 2 | 2.3 | 1 | 0 | 1 |
| NDF120172 | Black | 3 | 3 | . | 3 | 6 | 6 | . | . | . | 4.2 | 2 | 2 | 2 | . | . | . | . | . | . | 2 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 1 | 2 | 3 |
| NDF120174 | Black | . | . | . | . | . | . | . | . | . | . | 3 | 4 | 6 | 2 | 2 | 2 | 2 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 2 | 2 | 2.7 | 1 | 0 | 1 |
| NDF120175 | Black | 3 | 3 | 4 | 3 | 3 | 4 | 3 | 3 | 3 | 3.2 | 2 | 2 | 2 | 2 | 2 | 2 | . | . | . | 2 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 1 | 0 | 1 |
| NDF120178 | Black | 3 | 3 | 3 | 5 | 5 | 3 | 3 | 3 | 3 | 3.4 | 2 | 3 | 3 | 6 | 6 | 6 | 6 | 6 | 6 | 4.9 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 1 | 0 | 1 |
| NDF120180 | Black | 3 | 3 | 3 | 3 | 3 | 5 | 4 | 4 | 4 | 3.6 | 3 | 3 | 3 | 2 | 2 | 2 | . | . | . | 2.5 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 0 | 1 |
| NDF120181 | Black | 3 | 3 | 3 | 3 | 5 | 6 | . | . | . | 3.8 | 2 | 2 | 3 | 4 | 4 | 6 | . | . | . | 3.5 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 0 | 1 |
| NDF120185 | Black | 4 | 4 | 7 | 4 | 5 | 6 | . | . | . | 5 | . | . | . | . | . | . | . | . | . | . | 4 | 4 | 4 | 5 | 5 | 5 | 4 | 4 | 4 | 4.3 | 1 | 0 | 1 |
| NDF120187 | Black | 3 | 3 | 4 | 3 | 3 | 3 | 6 | 6 | 6 | 4.1 | 2 | 2 | 3 | 2 | 2 | 2 | . | . | . | 2.2 | 3 | 3 | 3 | 3 | 3 | 3 | . | . | . | 3 | 1 | 0 | 1 |
| NDF120191 | Black | 3 | 3 | 4 | . | . | . | - | . | . | 3.3 | 2 | 2 | 2 | 2 | 2 | 2 | . | . | . | 2 | 3 | 3 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 3.7 | 1 | 0 | 1 |
| NDF120208 | Black | 4 | 4 | 4 | 4 | 4 | 5 | 3 | 3 | 4 | 3.9 | . | . | $\cdot$ | . | . | . | . | . | - | . | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 1 | 0 | 1 |





| ID code | Class | 1st trial |  |  |  |  |  |  |  |  |  | 2nd trial |  |  |  |  |  |  |  |  |  | 3rd trial |  |  |  |  |  |  |  |  |  | Marker data |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | Plant 1 |  |  | Plant 2 |  |  | Plant 3 |  |  | Mean | Plant 1 |  |  | Plant 2 |  |  | Plant 3 |  |  | Mean | Plant 1 |  |  | Plant 2 |  |  | Plant 3 |  |  | Mean |  |  |  |
|  |  | L1 | L2 | L3 | L1 | L2 | L3 | L1 | L2 | L3 |  | L1 | L2 | L3 | L1 | L2 | L3 | L1 | L2 | L3 |  | L1 | L2 | L3 | L1 | L2 | L3 | L1 | L2 | L3 |  | SAP6 | SU91 | Both |
| 2125 LRK | LRK | 3 | 3 | 3 | . | . | . | . | . | . | 3 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | . | . | . | . | . | . | . | . | . | . | 1 | 2 | 3 |
| 2126 LRK | LRK | 3 | 3 | 3 | 3 | 4 | 4 | 3 | 3 | 3 | 3.2 | 3 | 5 | 6 | 3 | 3 | 3 | . | . | . | 3.8 | . | . | . | . | . | . | . | . | . | . | 1 | 0 | 1 |
| 2127 LRK | LRK | 4 | 5 | 6 | 3 | 3 | 3 | . | . | . | 4 | 2 | 2 | 2 | 2 | 2 | 2 | 3 | 3 | 3 | 2.3 | . | . | . | . | . | . | . | . | . | . | 1 | 2 | 3 |
| MONTCALM DRK | DRK | 3 | 3 | 3 | 3 | 3 | 4 | 2 | 2 | 2 | 2.8 | 2 | 6 | 7 | 3 | 3 | 3 | 2 | 2 | 3 | 3.4 | . | . | . | . | . | . | . | . | . | . | 1 | 0 | 1 |
| REDHAWK DRK | DRK | 3 | 3 | 3 | 3 | 3 | 4 | 3 | 3 | 8 | 3.7 | 2 | 2 | 3 | 2 | 2 | 2 | 2 | 2 | . | 2.1 | . | . | . | . | . | . | . | . | . | . | 0 | 0 | 0 |
| 1895 DRK | DRK | 3 | 3 | 5 | 3 | 3 | 4 | 4 | 4 | 4 | 3.7 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | . | . | . | . | . | . | . | . | . | . | 0 | 0 | 0 |
| 1900 DRK | DRK | 3 | 3 | 3 | x | X | X | 3 | 3 | 3 | 3 | 2 | 2 | 2 | . | . | . | . | . | . | 2 | . | . | . | . | . | . | . | . | . | . | 1 | 0 | 1 |
| 1930-D-DRK | DRK | 3 | 3 | 7 | 3 | 3 | 3 | 3 | 3 | 3 | 3.4 | 2 | 3 | 5 | 2 | 2 | 2 | 5 | 5 | 6 | 3.6 | . | . | . | . | . | . | . | . | . | . | 1 | 0 | 1 |
| 1934 DRK | DRK | 3 | 3 | 4 | x | X | x | 3 | 3 | 3 | 3.2 | 2 | 2 | 2 | 2 | 2 | 3 | 2 | 2 | 3 | 2.2 | . | . | . | . | . | . | . | . | . | . | 1 | 0 | 1 |
| 1941 DRK | DRK | 3 | 5 | 5 | 4 | 4 | 4 | 4 | 4 | 4 | 4.1 | 3 | 3 | 3 | 3 | 3 | 7 | . | . | . | 3.7 | . | . | . | . | . | . | . | . | . | . | 0 | 0 | 0 |
| 1944 DRK | DRK | X | X | X | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 2 | 2 | 2 | 2 | 2 | . | . | . | 2 | . | . | . | . | . | . | . | . | . | . | 0 | 0 | 0 |
| 1946 DRK | DRK | 4 | 4 | 5 | 4 | 4 | 5 | 5 | 5 | 5 | 4.6 | 2 | 2 | 3 | 5 | 5 | 7 | 3 | 3 | 3 | 3.7 | . | . | . | . | . | . | . | . | . | . | 0 | 0 | 0 |
| 1957 DRK | DRK | 2 | 2 | 2 | 2 | 2 | . | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 3 | 3 | 3 | . | . | . | 2.5 | . | . | . | . | . | . | . | . | . | . | 1 | 2 | 3 |
| 1959 DRK | DRK | 2 | 2 | 6 | 2 | 4 | 9 | . | . | . | 4.2 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | . | . | . | . | . | . | . | . | . | . | 1 | 2 | 3 |
| 1967 DRK | DRK | 2 | 2 | . | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 9 | 2.8 | . | . | . | . | . | . | . | . | . | . | 0 | 2 | 2 |
| 1971 DRK | DRK | 2 | 2 | 5 | 2 | 2 | 3 | 2 | 7 | 8 | 3.7 | 2 | 2 | 2 | 2 | 2 | 2 | . | . | . | 2 | . | . | . | . | . | . | . | . | . | . | 1 | 2 | 3 |
| 1972 DRK | DRK | 2 | 3 | 3 | 2 | 2 | 2 | 2 | 2 | 8 | 2.9 | 2 | 2 | 2 | 3 | 3 | 3 | . | . | . | 2.5 | . | . | . | . | . | . | . | . | . | . | 1 | 2 | 3 |
| 1978 DRK | DRK | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 5 | 9 | 3.1 | . | . | . | . | . | . | . | . | . | . | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 2 | 3 |
| 1988 DRK | DRK | 2 | 2 | 2 | X | X | X | . | . | . | 2 | 2 | 2 | 3 | . | . | . | . | . | . | 2.3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 4 | 4 | 3.2 | 0 | 0 | 0 |
| 1999-D-DRK | DRK | 3 | 4 | 4 | 3 | 3 | 3 | . | . | . | 3.3 | 2 | 2 | 2 | 7 | 7 | 7 | . | . | . | 4.5 | . | . | . | . | . | . | . | . | . | . | 0 | 0 | 0 |
| 2009 DRK | DRK | 6 | 9 | 9 | . | . | . | . | . | . | 8 | 4 | 5 | 7 | 6 | 9 | 9 | 6 | 8 | 9 | 7 | . | . | . | . | . | . | . | . | . | . | 0 | 0 | 0 |
| 2013 DRK | DRK | 4 | 5 | 9 | 9 | 9 | 9 | . | . | . | 7.5 | 5 | 5 | 8 | 5 | 5 | 5 | . | . | . | 5.5 | . | . | . | . | . | . | . | . | . | . | 0 | 0 | 0 |



APPENDIX C. PHENOTYPIC DATA OF COMMON BACTERIAL BLIGHT DISEASE SEVERITY IN FIELD AND GREENHOUSE CONDITIONS AND THE GENOTYPIC

DATA









