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A Genome Wide Association Study of Heat Tolerance in Snap Bean (Phaseolus vulgaris)

Morgan Stone
mstone9@clemson.edu

A GENOME WIDE ASSOCIATION STUDY OF HEAT TOLERANCE IN SNAP BEAN (*PHASEOLUS VULGARIS*)

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Morgan White Stone

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Dr. Brian Ward, Advisor

Dr. Sandra Branham, Co-Advisor

Dr. William Bridges

Dr. Richard Boyles

ABSTRACT

Bean production in the United States has decreased while the temperatures have been steadily increasing, reaching new highs each year. Heat stress is detrimental to common bean (also known as snap bean) production. Symptoms of heat stress include decrease in pollen viability, shriveling of pods, and pod abortion making them unmarketable. Pod production of 323 snap bean accessions from a large diversity panel was assessed in a randomized complete block design with field trials at two different times in the spring season. The results show a significant decrease in the number of pods produced per plant and weight of pods harvested in the heat-stressed planting date. Further, a genome-wide association study (GWAS) was conducted to identify markers associated with heat tolerance. We report accessions that were most productive under heat stress as well as the underlying quantitative trait loci (QTLs) associated with snap bean heat tolerance in this genome wide association study. Overall, there were 15 significant SNPs found across the number of pods and weight of pods yielded in the heat stressed environment. Of those significant SNPs in heat conditions, four encoded heat shock proteins.

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CHAPTER ONE

LITERATURE REVIEW

Introduction

Fabaceae is the third largest flowering plant family, with 20,000 distinct species and 625 edible species (Doyle and Luckow 2003, Ulian et. al 2020). One of the most economically important species of the family is *Phaseolus vulgaris,* or common bean, which is widely cultivated for dry beans and fleshy, edible pods.

Snap beans are referenced as several different names interchangeably, including French beans, garden beans, green beans, or haricot beans (USDA SNAP-Ed Connection 2023). The name 'snap bean' was given from the audible sound they make when bent to break off during harvest. The harvested product of a snap bean can be used for either the whole, fleshy pod (young/immature) or for the mature seed within the pod after the seed is dried out, which is then referred to as a dry bean.

Snap beans can be grown throughout the United States depending on the time of year. Optimal snap bean production nighttime temperatures range from 18°C-21°C and daytime temperatures from 21°C-27°C (Rainey and Griffiths 2005). Temperatures exceeding higher than 30°C during the day or higher than 20°C at night have been reported to impact bean yields (Rainey and Griffiths 2005, Sita et al. 2017, Vargas 2021). Snap beans do not tolerate frost; therefore, planting must be after the last spring frost with soil temperatures above 15°C (Venter 2021). Mexico (23.6345° N, 102.5528° W) and

Morocco (31.7917° N, 7.0926° W) (temperate and subtropic environments) tend to be the leading countries that can produce marketable pods during their winter months for export to countries during their off seasons (Venter 2021). Snap beans do well in slightly acidic soil (5.8-6.6 pH) with good drainage. USDA Hardiness zones for snap bean growth range from 6-11.

Importance of snap bean

Snap beans are an important crop not only due to their ability to grow in diverse soil and climatic conditions throughout the world, but for the revenue brought to countries around the world. China produces 76.7% of the world's snap beans (Venter 2021). As of 2022, snap bean production around the world was 25 million tons (USDA NASS 2023). The United States is the third largest producer of snap beans, averaging 864,656 tons annually over a five-year period that ended in 2019 (Venter 2021). Across the United States, snap beans were planted on 164,600 acres in 2022 for an annual value of $325M, with more than 20% of that production in the Southeast (USDA NASS 2023). Wisconsin, the leading producer of snap beans, accounts for over 50% of the production in the United States. Of the $325M value in 2022, $201M was from fresh market snap beans and $124M came from processed snap beans (USDA NASS 2023). The same year utilized processed (canned and frozen) beans weighed 601,673 tons, while the utilized fresh market beans weighed 123,710 tons and were more profitable (USDA NASS 2023). In 2018, utilized production of processed beans was 659,113 tons (USDA NASS 2021). Data from 2019-2022 shows other crops (sweet corn, carrots, cantaloupe) also have a slight production decrease during that period of the global pandemic (USDA NASS 2021 and 2023). The United States imports and exports fresh, frozen, and preserved snap beans. Mexico makes up 68.5% of volume from all imports to the United States for fresh beans (USDA Economic Research Service 2022). Canada is the top importer of frozen and preserved beans from the United States (USDA Economic Research Service 2022). Snap bean prices have been steadily increasing each year from $13.1/cwt in 1970 to $67.20/cwt in 2021. (USDA Vegetable and Pulse Yearbook 2022, USDA Economic Research Service).

Snap beans have important benefits to human health through high levels of nutrients and vitamins. These vibrant pods are an excellent source of dietary fiber, providing around 2.6 grams per serving, which supports digestive health and may reduce the risk of heart disease (USDA SNAP-Ed Connection 2023). Green beans are also rich in vitamins and minerals, including vitamin C, vitamin K, and folate (Beebe 2012). Snap beans contain small amounts of essential minerals such as potassium, iron, and magnesium, which contribute to various physiological functions in the body (Beebe 2012).

Snap beans provide nutrients back into the soil, as do many other legumes. Root nodules form on the plants through interaction with bacteria found in the soil, called Rhizobia (Peix et al. 2014). This symbiotic relationship with Rhizobia, nitrogen-fixing bacteria, converts atmospheric nitrogen into plant-available organic nitrogen (Peix et al. 2014). In return, the plant provides the rhizobia with essential nutrients and a favorable environment for their growth. This relationship can reduce reliance on synthetic fertilizers, foster healthier plant growth, improve yield, and contribute to the overall fertility and productivity of the soil. As leguminous plants, they have the ability to suppress weeds, reduce erosion, and improve soil structure through their root systems (Uebersax et al. 2022). Crop rotation of beans and small grains are often recommended for sustainable soil management (Uebersax et al. 2022). Native Americans adapted a different system for sourcing food and sustaining the land for over 3,500 years which included beans (Ngapo et al. 2021). The intercropping method is called The Three Sisters, which are comprised of corn, beans, and squash planted together (Ngapo et al. 2021).

In summary, recognizing the importance of snap beans from a consumer or grower perspective can benefit the environment in three major ways. Snap beans can promote biodiversity in farming systems, supply nitrogen fixation benefits that can reduce the application of chemical fertilizers to the soil, and incorporate plant-based proteins in the human diet that, in comparison to animal sourced proteins, are less energy and resource intensive to produce with often reduced negative effects on the environment (Vasconcelos et al. 2020).

Genetic diversity and population structure of common bean

There are two major ancestral groups of origin, Middle American (Central America) and Andean (South America). These two gene pools can further be categorized into seven subgroups or races based on their differences in leaf morphology, seed size, seed shape, pod morphology, stem thickness, internode length, plant habit, allozyme type, and phaseolin type (Hao 2023, Wallace 2018). The Middle American gene pool can be separated by its genetic diversity into the Mesoamerican, Durango, Jalisco, and Guatemala races (Hao 2023, Wallace 2018). The Andean gene pool can be classified into three races, including Nueva Granada, Peru, and Chile (Hao 2023, Mensack et al. 2010, Wallace 2018). DNA analysis suggests that common beans first originated in Ecuador and Peru (Kelly 2010). Common beans were domesticated and introduced into the United States 5,000 years ago (Kelly 2010).

Common beans are grouped into several market classes depending on the region, color, and size of seed. Common beans, *P. vulgaris*, can refer to green beans, anasazi beans, navy beans, black beans, northern beans, kidney beans, pinto beans, and cannellini beans. There is also the difference in use of dry bean and fresh bean. Typically, fresh snap beans will have thick, fleshy pod walls and are low in fiber (Wallace 2018). Common beans have both stringy and stringless types, in which scientists believe genetic control of strings is independent of pod wall fiber (Wallace 2018). Common bean market classes were grouped into races through DNA analysis and seed characteristics. Through genetic evaluation, multiple bean classes or types overlap across subgroups (Blair et al. 2009). To summarize bean types into regions, scientists have discovered many navy and black beans are from the Mesoamerican race (1), pinto beans are from the Durango race (2), small red and pink beans are the Jalisco race (3), climbing beans are the Guatemala race (4), kidney beans, bush cranberry beans, and a majority of snap beans are the Nueva Granada race (5), yellow beans, bush and climbing beans belong to the Peru race (6), and vine cranberry beans are the Chile race (7) (Beebe et al. 2000, Blair et al. 2009, Kelly 2010, Mensack et al. 2010). Depending on regional domestication, cultivars can have geographical and ecological adaptations. For example, Mesoamerica genotypes are often adapted to the warmer and more humid environments of southern Mexico and Central America as opposed to higher altitudes in the Andes (Singh et al. 1991).

From these two races, diversity panels have been created to examine genetic makeup and further study the similarities or differences in the genomes from Europe, China, and North America. The Common Bean Coordinated Agriculture Project panel, otherwise known as, BeanCAP has been genotyped using Illumina Infinium Genechip BARCBEAN6K_3 platform (Myers 2021). Additional panels include The Middle American Diversity Panel (MDP) (Moghaddam et al., 2016), Andean Diversity Panel (ADP) (Cichy et al., 2015), and The Snap Bean Diversity Panel (SBDP) containing both Mesoamerican and Andean accessions, which have been utilized for association mapping and characterization of specific agronomic traits. The Snap Bean Association Panel (SnAP) is a diversity panel which consists of a total of 378 cultivars, comprising 150 accessions from the Bean CAP SBDP and an additional 228 accessions that were released/expired from Plant Variety Protection (PVP) (USDA-AMS, Myers and Celebioglu 2023).

Snap bean physiology

Cultivated snap beans are categorized by two growth types, pole beans and bush beans. Pole beans are tall with viny stems and require a trellis structure to support their growth and prevent diseases from extended soil contact. These traits are mainly inherited by wild common beans (Kwak et al. 2012). Bush beans are short and do not require trellising, which makes them better suited for large-scale commercial production especially for the purpose of mechanical harvest (Kwak et al. 2008). Differences in inflorescence period also define these two types of snap beans (Kwak et al. 2012). Indeterminate (pole) beans will flower over a longer period and produce pods over a longer span of time, whereas determinate (bush) beans flower quickly and set pods over a shorter time span. Flowering times are not photoperiod sensitive in domesticated snap beans but are short-day plants in their wild progenitors (Kwak et al. 2008). For determinate beans, the production of modules, which includes a subtending internode, a leaf, and an inflorescence in the axil of the leaf, typically has reached development by the fifth trifoliate (Kwak et al. 2012). Wild, indeterminate types differ in growth habit, because new modules will continue to produce until senescence (Kwak et al. 2012). Snap beans take approximately 30 days to reach flowering and 60 days to produce mature pods (OCED 2016).

Effects of heat stress on pod production

Heat stress during floral initiation and development can have significant negative impacts on snap bean pods and production, decreasing the yield, quality, and marketability of the crop. Snap beans are sensitive to high temperatures during their flowering period, and prolonged exposure to heat stress can lead to various physiological and biochemical changes that negatively influence pod development, overall yield and quality.

Heat stress during flowering and pod development stages can disrupt normal pollination and fertilization processes, leading to poor pod set and reduced pod development. Flower abscission of reproductive organs can result in smaller and fewer pods produced on the plants (Rainey and Griffiths 2005, Ofir et al. 1993). Even when heat susceptible plants set pods, the quality of pods may be tough, fibrous, less flavorful, or pods may lose their vibrant color or be curved in shape. Heat-stressed snap beans may not meet the desired standards for size, color, texture, and flavor that consumers and buyers are looking for which in turn can have a significant impact on the marketability and profitability of snap beans.

When beans experience heat stress, they undergo a range of chemical responses, and certain metabolites are expressed or accumulated as part of their adaptive mechanisms. These responses aim to protect the plant from damage caused by high temperatures and maintain cellular homeostasis (Zhao et al. 2020). Some of the key plant responses and metabolites expressed during heat stress in beans include:

1. Heat Shock Proteins (HSPs): Heat stress triggers the synthesis of heat shock proteins, which act as molecular chaperones, assisting in protein folding and preventing the aggregation of denatured proteins (Bita and Gerats 2013). HSPs play a crucial role in protecting the plant's proteins and maintaining their

functional integrity under heat stress conditions (Simões-Araújo et al. 2003, Mallick et al. 2022).

2. Reactive Oxygen Species (ROS) Scavengers: Heat stress can lead to the production of reactive oxygen species (ROS), which are harmful molecules that can damage cellular components (Bita and Gerats 2013, Zhao et al. 2020). Beans respond to heat stress by upregulating the production of antioxidants such as ascorbic acid (vitamin C), glutathione, and superoxide dismutase (SOD), which help neutralize ROS and minimize oxidative damage (Maalouf et al. 2022, Sita et al 2017, Zhao et al. 2020).
3. Proline and Other Osmolytes: Beans accumulate osmolytes such as proline, betaine, and trehalose as compatible solutes in response to heat stress. These osmolytes act as osmoprotectants, helping to maintain cellular water balance and stabilize protein structures during heat stress (Sita et al. 2017, Zhao et al. 2020).
4. Phytohormones: Heat stress can lead to alterations in the levels of various phytohormones, including abscisic acid (ABA), ethylene, and jasmonic acid. These hormones play regulatory roles in stress responses, including stomatal closure, gene expression, and defense mechanisms (Sita et al. 2017).
5. Secondary Metabolites: Under heat stress, the biosynthesis of certain secondary metabolites may be induced in beans. These compounds, such as flavonoids and phenolic compounds, can act as antioxidants and participate in defense mechanisms against oxidative stress (Maalouf et al. 2022, Sita et al. 2017).

The specific responses and metabolites expressed in beans during heat stress likely vary depending on the severity and duration of the stress, as well as the genetic background and environmental conditions. Understanding these responses and metabolic changes is essential for developing strategies to enhance heat tolerance in beans and other crops.

In summary, heat stress during reproductive development negatively affects snap bean pods and production by reducing pod development, accelerating senescence, incomplete pod filling, causing poor pod quality, and decreasing overall yield. These adverse effects can impact the marketability of snap beans, as the heat-stressed crop may not meet consumer preferences and quality standards. Development of new heat-tolerant snap bean varieties is necessary to mitigate the effects of heat stress on snap bean crops. These varieties may have improved photosynthetic capabilities, which could deliver better root and shoot growth and yield production or begin to flower earlier to reduce exposure to heat (Langstroff et al. 2022).

Cultivar development for heat tolerance

Several heat tolerant cultivars have been released in recent years, but most are dry beans. In 2007, a dry bean cultivar named ‘Verno’ was released with multiple disease resistance and adaptation as a high temperature–tolerant cultivar (Beaver et al. 2008). University of Puerto Rico Agricultural Experiment Station and the USDA-ARS reported ‘Verno’ will improve yield and seed quality of green-shelled beans produced in Puerto Rico (Beaver et al. 2008). ‘Bella’ is a white-seeded common bean registered for multiple diseases resistance and tolerance to high temperatures with low fertility soil (Beaver et al. 2018). Black bean lines, 'TARS-MST1' and 'SB-DT1' were developed by the USDA-ARS, the University of Nebraska Agricultural Research Division, and the University of Puerto Rico Agricultural Experiment Station for their tolerance to high ambient temperature and drought stress and resistance to root rot and common bacterial leaf blight (Porch et al. 2012). Shonnard and Gepts (1994) identified the heat-tolerant Type I kidney bean, 'G122'. This study highlighted quantitative inheritance for bud retention and pod fill under heat stress, revealing significant additive genetic variability and cytoplasmic effects (Shonnard and Gepts 1994). Bud retention results showed significant dominance effects in the accession and could be beneficial for increasing yield (Shonnard and Gepts 1994). Porch and Jahn (2001) used 'G122' as well for a study with 'A55' line to examine microsporogenesis of anther indehiscence and pollen viability under high temperatures (Porch and Jahn 2001).

'Haibushi' is another heat tolerant cultivar that has been tested in comparison to 'Kentucky Wonder', a heat susceptible cultivar (Kumar et al. 2005). Parental crosses of a heat-tolerant snap bean line, 'Cornell 503', and heat-sensitive line, 'Majestic' were tested in a reproductive development study (Rainey and Griffiths 2005). 'Cornell 503' experienced a 17% increase in pod number during the moderate heat treatment, whereas 'Majestic' had a 29% decrease (Rainey and Griffiths 2005). In the next heat intensity level of 33°C/30 °C, 'Cornell 503' had a 21% decrease and 'Majestic' had a 100% decrease in yield compared to the non-stressed environment. Rainey and Griffiths (2005) published another study that same year in which they evaluated 24 common bean accessions under three different heat treatments. Genotypes were assessed for pod yield, seed number, and seed weight under heat stress conditions (Rainey and Griffiths 2005). ‘Carson’ and ‘HT38’ had an increase in pod yield under heat-stressed conditions of 30°C/27°C (Rainey and Griffiths 2005). A heat susceptibility index was formulated in which ‘Hystyle’ produced stable yields under heat stress (Rainey and Griffiths 2005).

Genetic studies on heat tolerance

Before diving into genetic studies, it is important to note that snap beans are self-pollinating diploids (2n=22). Again, few studies have been done on heat tolerance in snap beans and even fewer with a large diversity panel. A quantitative trait loci (QTL) mapping study used a recombinant inbred line (RIL) dry bean population of Indeterminate Jamaica Red (IJR) by AFR298 of the Andean gene pool to evaluate heat stress (Vargas et al. 2021). This study mapped multiple traits such as days to flower, pod harvest index, pollen viability, pod number, seed weight. Vargas et al. (2021) found chromosome associations with Pv01, Pv04, and Pv09 for days to flower in heat while chromosome Pv05 was significantly associated with pollen viability and pod harvest index traits. In addition, a QTL on chromosome Pv08 was associated with improved pollen viability and yield (Vargas et al. 2021).

In a 2019, a GWAS focused on production traits in abiotic stressed environments in a diversity panel of primarily Mesoamerica dry bean accessions, found that SNP Phvul.003G187400 on chromosome Pv03 was linked to yield under heat stress (Oladzad et al. 2019). Additionally, they discovered three other SNPs, one at Pv08 and two at Pv11, that accounted for 20% of the variation in yield under heat stress (Oladzad et al. 2019). In the same study they evaluated flowering times and found a major QTL peak at Pv03 (Phvul.003G181900) (Ozladzad et al. 2019). In another GWAS with 78 common bean accessions, heat stress indices were used to identify 120 significant genome-environment associations across the genome (Lopez-Hernadez and Cortes 2019).

A study on common bean pod maturation discovered candidate genes from analyzing the transcriptomes of five developmental pod stages, from pod setting to maturation in two nuña bean accessions, PMB0225 and PHA1037 (Gomez-Martin et al. 2020). Pod maturation relies upon complex gene expression changes, which in turn are important for seed formation and dispersal (Gomez-Martin et al. 2020). Differentially expressed genes (DEGs) found in this study for modulating pod maturation could be manipulated through molecular breeding to develop strategies with heat stressors to improve yield and pod quality of common bean crops (Gomez-Martin et al. 2020).

The knockout gene SlMAPK3 was discovered in tomato in response to heat stress (Yu et al. 2019). In *Arabidopsis thaliana*, scientists discovered a pollen-specific Cyclic Nucleotide-Gated cation Channel 16 (cngc16), expressed in plant reproduction under temperature-stress conditions (Ishka et al. 2018). Chickpeas, though cool-season legumes, have also been evaluated for their heat tolerance, showing analogous responses to heat stress in terms of yield losses attributed to flower and pod abortion (Jha et al. 2021). Thudi et al. (2017) conducted a heat trial similar to ours by doing two sowing dates, a normal sowing time and a late sowing time, in three replicates. From this drought and heat study 113 gene-based SNPs were identified. Five candidate genes from significant SNPs associated with abiotic stress and yield and/or flowering traits, ERECTA, abscisic acid stress and ripening (ASR), aminoaldehyde dehydrogenase (AMADH), CAP2 promoter, and dehydration responsive element binding protein (DREB).

Tepary bean (*Phaseolus acutifolius*) breeding potential

There are four other cultivated Phaseolus species in addition to *P. vulgaris*, *P. dumosus* (year bean), *P. coccineus* (scarlet runner), *P. lunatus* (lima bean), and *P. acutifolius* (tepary bean) (Delgado-Salinas et al. 2006). As closely related species, tepary beans can be successfully hybridized through controlled pollination and introgression (Gujaria-Verma 2016). Tepary bean *(P. acutifolius)* is a remarkable legume that originated in the arid regions of the Americas, particularly in the southwestern United States and northern Mexico (Kelly 2010, Wolf 2018). It has evolved to thrive in hot and dry environments, exhibiting exceptional drought and heat tolerance (Rainey and Griffiths 2004, Wolf 2018). Tepary beans possess various adaptive traits, including deep root systems that efficiently scavenge water from the soil, allowing them to endure prolonged periods of drought (Wolf 2018). These beans also have a waxy cuticle on their leaves, reducing water loss through transpiration, further enhancing their resilience in arid conditions (Gujaria-Verma et al. 2016). These traits hold great promise for improving the resilience of other crops to water scarcity and high temperatures, making tepary bean a valuable genetic resource for future agricultural sustainability and climate adaptation.

However, it is important to note that successful hybridization between these two species may require careful laboratory or greenhouse procedures due to potential barriers in natural cross-pollination. Researchers and plant breeders often use specific breeding techniques and methods to overcome these challenges and create successful hybrids between the two species. Journals have reported that successful crosses with tepary bean require an embryo rescue step to produce viable hybrids (Kelly 2010, Rao et al. 2013). In the past, these crosses have been used to introgress disease resistance from tepary bean into common bean (Moghaddam et al. 2021). The hybridization of these two *Phaseolus* species can lead to the creation of new genetic combinations, potentially resulting in hybrid offspring with unique traits and characteristics from both parental species. This process is valuable in plant breeding programs as it allows the introduction of desirable traits from one species into another, expanding genetic diversity and improving the overall adaptability and performance of the resulting hybrids.

Genome-Wide Association Study (GWAS)

Genome-wide association studies (GWAS) have revolutionized the field of plant genetics by enabling researchers to evaluate the genetic basis of quantitative traits in collections of unrelated accessions and understand their complex interactions with the environment. The history of GWAS with plants dates to the early 2000s when advances in high-throughput genotyping technologies allowed the examination of thousands to millions of genetic markers across plant genomes (Cortes et al. 2021). GWAS is an evaluation of the association between each genotyped marker and the phenotypic trait of interest that has been measured across a large sample size (Korte and Farlow 2013). By using mixed model analysis, one can find marker associations with SNPs and estimate the genetic variation of the phenotypic trait (Wang and Zhang 2021).

One of the commonly used packages for GWAS in plants is the Genome Association and Prediction Integrated Tool (GAPIT) (Wang and Zhang 2021). GAPIT is a powerful and user-friendly software package that efficiently performs GWAS by incorporating both population structure and kinship information to control false positive associations (Lipka et al. 2012). By accounting for population structure, which represents the genetic differences between subgroups in the plant population, GAPIT helps in identifying true genetic associations rather than false positives caused by population stratification (Lipka et al. 2012, Wang et al. 2014). Additionally, the inclusion of kinship allows for the inclusion of genetic relatedness among individuals, thus improving the accuracy of association results (Wang and Zhang 2021).

GAPIT integrates multiple GWAS models into one platform, including a mixed linear model (MLM; Lipka et al. 2012), fixed and random model circulating probability unification (FarmCPU; Liu et al. 2016), and Bayesian-information and linkage-disequilibrium iteratively nested keyway (BLINK; Huang et al. 2019) for each trait of focus (Wang and Zhang 2021). MLM is a single locus test, while FarmCPU and BLINK are multi-locus tests (Wang and Zhang 2021). MLM uses all markers to derive kinship from the individuals and traits of interest, whereas FarmCPU finds pseudo quantitative trait nucleotides (QTNs) to develop kinship (Wang and Zhang 2021). Statistical power differs among the three different models.

The power of GWAS to identify a true association between a SNP and trait is dependent on the phenotypic variance, which is determined by how strongly the two allelic variants differ in their phenotypic effect and their frequency in the sample (Korte and Farlow 2013). The power to detect is improved when the sample size is increased to recover meaningful associations (Korte and Farlow 2013). Often significant associations are detectable because causative SNPs or structural variants are in sufficient linkage disequilibrium (LD) with genotyped markers (Korte and Farlow 2013). LD is a non-random co-occurrence of two or more alleles that occurs naturally between loci in close radius (Korte and Farlow 2013). False discovery rate (FDR) can be an informative principle when determining the performance of a GWAS based on the genetic architecture of a trait or for identifying candidate loci (Korte and Farlow 2013). GWAS has been instrumental in identifying genetic variants associated with important agronomic traits such as yield, disease resistance, and drought tolerance (Alseekh et al. 2021, Arkwazee et al. 2022, Cortes et al 2021, Hart and Griffiths 2015, Raggi et al. 2019, Tafesse et al. 2020).

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CHAPTER TWO

A GENOME WIDE ASSOCIATION STUDY OF HEAT TOLERANCE IN SNAP BEAN (*Phaseolus vulgaris*)

Introduction

Snap bean annual production is valued at $325 million dollars in the United States, with 160,000 acres planted each year (USDA, National Agricultural Statistics Service 2022). The predominant regions for snap bean production in the United States include Wisconsin, New York, Florida, and Michigan (USDA, NASS 2022). Snap bean pod production is hindered under heat stress during the flowering period when air temperatures exceed 30ºC day/20ºC night (Suzuki 2001, Rainey and Griffiths 2005, Vargas et al. 2021). Heat stress can impair several physiological processes that are linked to seed size and quality (Janni et al. 2020). Symptoms of heat stress include pollen sterility, flower abscission, malformation of pods, and embryo abortion or seed desiccation. The National Oceanic and Atmospheric Administration (NOAA) reports that, within the past 143 years, the top nine warmest years occurred exclusively from 2014 to 2022 (NOAA 2022). This escalating trend in annual atmospheric temperatures highlights an urgent need for research aimed at developing heat-tolerant snap bean cultivars.

Researchers have studied abiotic stresses in common beans and other closely related crops (Janni et al. 2020). A commonality across these crops is a decrease in yield as temperatures increase, which can be attributed to flowering times, pollen viability and pod fill (seed weight) (Monterroso and Wien 1990, Oladzad et al. 2019, Raggi et al. 2019, Janni et al. 2020, Hassan et al. 2020, Vargas et al. 2021). Multiple studies of heat tolerance in common bean have been reported, but most have focused on dry bean germplasm (Blair and Beebe 2006, Beebe et al. 2013, Oladzad et al. 2019, Vargas et al. 2021). A GWAS of 78 wild bean accessions was screened and found five accessions tolerant to heat stress based on three different bioclimatic indices (López-Hernández and Cortés 2019). A total of 120 loci in 15 models were associated with the three heat stressed bioclimatic indices (López-Hernández and Cortés 2019). A GWAS of 192 common bean genotypes from landraces and single seed descent discovered seven candidate genes for flowering times (Raggi et al. 2019). A QTL mapping study using a dry bean recombinant inbred line (RIL) population with Indeterminate Jamacia Red as the heat tolerant parent identified two QTL on chromosomes Pv05 and Pv08 (Vargas et al. 2021). There have been several dry bean cultivars released for heat tolerance, including 'Verno' (Beaver et al. 2008), 'Bella' (Beaver at al. 2018), 'TARS-MST1' and 'SB-DT1' (Porch et al. 2012). Heat tolerant snap bean breeding lines 'Cornell 502' and 'Cornell 503' were released twenty years ago (USDA-REEIS, Rainey and Griffiths 2005).

Most common bean heat tolerance studies evaluated dry bean germplasm and the few reported for snap bean have been limited in terms of numbers of accessions and markers. Here we evaluated 266 accessions of the Snap bean Association Panel (SnAP) and 57 commercial cultivars for flowering and pod production traits in two years of field trials under ideal and heat-stressed conditions. Genotyping-by-sequencing (GBS) of the panel resulted in 28,978 SNPs, which were used for a genome-wide association study (GWAS) of days to flower, number of pods per plant, and the weight of pods per plant.

The objectives of this research were to identify (1) accessions with higher pod production than the commercial cultivars under ideal and/or heat-stressed conditions, (2) genomic regions associated with flowering and pod production and (3) the most promising candidate genes.

Materials and Methods

2.1. Plant materials

The Snap Bean Association Panel (SnAP) consists of a total of 378 accessions; comprising of 150 accessions from Common Bean Coordinated Agriculture Project (Bean CAP) Snap Bean Diversity Panel (SBDP) and an additional 228 historical cultivars that were released/expired from Plant Variety Protection (PVP) (USDA-AMS, Myers and Celebioglu 2023). Accession information was collected from the USDA Germplasm Resource Information Network (Supp. Table 1). Seeds for the SnAP were provided through in-kind support by Seneca Food Corporation (Marion, New York). Field evaluations of the SnAP were limited to 266 determinate bush types, including green beans, yellow wax beans, purple-podded beans, and Romano beans (Supp. Table 1). Fifty-seven commercial cultivars were included in the field trials to compare pod production of the historical accessions of the SnAP to the production range of modern cultivation.

2.2 Experimental location and design

Field trials were conducted at Clemson University's Coastal Research and Education Center in Charleston, SC (lat. 32º 47'34.5" N, long. 80º 04'14.1" W). The research plots consist of Yonges Fine Sandy Loam soil, with an organic matter content of approximately 1.25% and a pH of 6.75 (Clemson Agricultural Services Laboratory, Clemson, SC, National Cooperative Soil Survey U.S.A).

Evaluation of the SnAP was completed using a randomized complete block design (RCBD) with three replications and two planting dates, repeated over two consecutive years (2021-2022). The first planting dates in the ideal planting conditions (IPD) for snap beans were April 18th, 2021 and April 20th, 2022. The second planting dates were later in the season to induce heat-stress conditions (HPD) during flowering and were planted on May 18th, 2021 and May 18th, 2022. There were 323 lines in the first year, including 266 SnAP accessions and 57 cultivars. The number of SnAP accessions decreased to 256 in the second year due to lack of seed and fewer cultivars (*n*=22) included to reduce labor needs. Each plot was manually sown in a single row, with 20 seeds per accession, approximately 2.5 cm deep and 7.6 cm apart. The plots were 1.5 m in length with 0.9 m within row spacing between plots. The standard snap bean commercial cultivar, 'Caprice' (Harris Moran, Davis, CA), commonly grown throughout the southeastern U.S., was planted throughout the RCBD as a repeated check among the other accessions.

Standard field management practices were performed, including drip irrigation when needed, hand weeding, pesticide and herbicide applications, and fertilization. Over the course of the field study, a total of 53.3 cm of precipitation was documented during the 2021 trial period and 45.7 cm from April to July in 2022.

2.3 Harvest and storage

Snap beans, on average, take 60 days (seven to eight weeks after sowing) to reach harvest maturity (OCED 2016). Maturity was determined by sieve size and seed fill, ranging from 4-12 seeds with each seed approximately 1 cm in length (OCED 2016). Sieve size is the diameter of the pod. Harvest will typically begin when 50% of the pods are sieve size 4 (8.5-9.7 mm). Five plants from the center of each plot were harvested and stored in a paper bag. Plants were stored in a cooler maintained at 4.4°C to prolong shelf life during time needed to record all phenotypic traits (Kibar and Kibar 2019).

2.4 Phenotyping

A variety of phenotypic traits are important for pod marketability, including color, sieve size, and curvature. Given the time and labor constraints of the large-scale of this study, we directed our focus on three specific traits: flowering time, pod yield (measured by the number of pods per plant), and weight of pods per plant.

Flowering dates were recorded for each plot as the number of days from seeding to the first flower reached anthesis. We abbreviated this phenotype as ‘DTF’ (days to flower). Pods were systematically harvested from five representative plants within the central area of each plot. The total pod count was divided by the number of plants sampled, denoted as 'PPP' (pods per plant). The cumulative pod weight was measured and then divided by the number of plants sampled to calculate the variable 'KPP' (kilograms per plant). In cases where germination rates were suboptimal or low, resulting in fewer than five plants available for harvest, all viable plants were included in the measured sample. The number of plants used to calculate PPP and KPP was reflected accordingly.

2.5 Genotyping

Variants were called from genotyping-by-sequencing (GBS) data generated at Cornell University (Ithaca, NY) using Illumina HiSeq 2500 to produce 100 base-pair single-end reads. Reads were mapped to the *Phaseolus vulgaris* v2.1 genome, downloaded from Phytozome v13 (https://phytozome-next.jgi.doe.gov/info/Pvulgaris_v2_1). *Phaseolus vulgaris* v2.1 alignment removed any likely errors of overlapping SNPs with non-matching allele calls (DOE-JGI, USDA-NIFA). This latest version was filtered using VCFtools with a minor allele frequency (MAF) set greater than 0.05 to retain high-quality bi-allelic SNPs (Danacek et al. 2011).

2.6 Data analysis

In R software, standard statistical analysis was performed, including Analysis of Variance (ANOVA) of phenotypic data for combined years. Pearson's correlations were calculated between trial years and traits. Histograms for figures were created with the ggplot2 package using the statistical software R (R core team 2022). Tukey's Honest Significance Difference (HSD) test was calculated using R to compare the means of traits. Best Linear Unbiased Estimators (BLUEs) were calculated for the phenotypic data of each accession using the lmer function (lme4 package) to incorporate fixed (genotype) and random effects (year, planting date, rep, field row, year within treatment, and genotype within rep) as a mixed-effects model in R (Bates et al. 2015). The Genome Association and Prediction Integrated Tool (GAPIT) function from the GAPIT3 package was installed in R software (Wang and Zhang 2021) and used with Palmetto, a supercomputer cluster built and maintained by Clemson University (Clemson Palmetto 2023) to compute rapid results. GAPIT was used to identify significant SNPs with the false discovery rate (FDR) set at <0.01 (see Table 3). The GWAS models created in GAPIT included the mixed linear model (MLM), fixed and random model circulating probability unification (FarmCPU), and Bayesian-information and linkage-disequilibrium iteratively nested keyway (BLINK) for each trait across the two years in separate planting dates (Huang et al. 2019, Lipka et al. 2012, Liu et al. 2016, Wang and Zhang 2021). The optimal number of principal components (PCs) were chosen by using the model.selection=TRUE option within GAPIT and selecting the model with the highest Bayesian information content (BIC) from each trait to account for population structure in the GWAS (Wang and Zhang 2021). The intervals for selection of candidate genes around each significant SNP was determined by genome-wide levels of linkage disequilibrium (LD) decay. LD decay rate of the collection was defined as the chromosomal distance where the average pairwise correlation coefficient (r^2) dropped to half its maximum value (Huang, et al. 2010). An interval of 1 million base pairs (bp), with 500,000 bp upstream and downstream of each significant SNP was used to search for heat shock proteins and other functionally relevant proteins that may promote heat tolerance.

Results

3.1 Phenotypic analysis

Phenotypic traits recorded in this study to determine heat tolerance were days to flower (DTF), pods per plant (PPP), kilograms per plant (KPP). Table 1 and Fig. 1 illustrate the average PPP for ideal planting dates (IPD) at 27.6 pods, while the heat-induced planting dates (HPD) averaged 14.2 pods, representing a 48.75% decrease from IPD. This notable reduction in pod production due to heat stress was observed between the months of May to July (Fig. 3).

Mean temperatures during the IPD were 28°C/18°C day/night, respectively, over the two-year study (Fig. 2). Although some days exceeded the optimal growth temperatures (27°C/21°C), they rarely surpassed 30°C, a threshold known to hinder common bean productivity during the flowering period (Rainey and Griffiths 2015). During HPD, temperatures averaged 31°C/23° C day/night, with the highest recorded temperature during the trial reaching 37°C (99°F). Fig. 4 illustrates line plots of combined years of Fig. 2 and Fig. 3 with daytime and nighttime temperatures. It can be noted that only four days in the HPD interval fell below the daytime (max) threshold of optimal growth temperatures, therefore heat-stress was successfully induced (Fig.4).

Several accessions, including ‘Oregon 1604M’, ‘Rapids’, and ‘Tendergreen’, have the earliest flowering times at 31 days in IPD. A total of 38 accessions flowered in 32 days. Notably those accessions with 32 DTF were ‘Blue Knight’ (53.4 PPP), ‘Sinclair Butterwax’ (51.8 PPP), and ‘Rapids’ (48.6 PPP) that demonstrated a higher PPP on average. ‘Blue Knight’ was top of the list for the other phenotypic trait used to assess yield, by producing the highest weight at 0.42 KPP. The top-performing accessions for PPP in IPD were ‘Roller’ from year one with 69.6 PPP and ‘Minuette’ from year two with 71.6 PPP. ‘Baby Bop’ had the top two-year mean of 48.9 PPP in IPD (Table 2).

The earliest flowering times observed from HPD were ‘Blue Knight’ and ‘Wrangler’ at 28 DTF, four days earlier than IPD accessions. A total of 33 accessions first flowered within 28 days after sowing. ‘Blue Knight’ and ‘Wrangler’ both had early DTF and production of over 40 PPP in the second year of the study. Another accession that demonstrated noteworthy pod production in HPD was ‘DMC 04-61’, which produced the most pods (63.2 PPP) with a weight of 0.24 KPP. ‘Blue Knight’ had the heaviest weight at 0.25 KPP with 42.4 PPP from HPD . However, despite the accessions that maintained above average pod production during HPD, the weight of KPP from the top accession in HPD was nearly half compared to IPD. In the second planting trial with heat induced conditions, some accessions exhibited very leafy and dense foliage but produced no pods.

Overall, ‘Flavor Sweet’ maintained high pod production for multiple reps and scored most significant in PPP under HPD conditions based on a Tukey’s HSD test (Table 3). There was moderate correlation in PPP between years under heat stress (r = 0.48) (Table 1). Across all the traits, there were moderate to high heritability for accessions to pass on these traits to offspring. There was a slight decrease in heritability under heat stress conditions (Table 1). The phenotypic trait of DTF was more heritable in the IPD (0.89) compared to HPD (0.71), as well as in comparison to the other two traits of PPP (0.67 IPD; 0.57 HPD) and KPP (0.64 IPD; 0.62 HPD) (Table 1).

3.2 Comparison of accessions to commercial cultivars

In both ideal and heat stress environments, ‘Flavor Sweet’ and ‘Dandy’ were among the top PI accessions for PPP (Table 2 and 3). Overall, 25 PI accessions had a higher HSD means than the highest pod-yielding cultivar, ‘HM6401’, which produced 35.87 pods in ideal conditions. As mentioned above, in the IPD, ‘Baby Bop’ stood out in its own significant group, boasting a remarkable average of 48.93 PPP, followed by another PI, ‘Dandy’ with a robust average of 47.13 PPP.

For the heat stress trial, ‘Flavor Sweet’ averaged 31.17 PPP and ‘Dandy’ averaged 29.7 PPP, which both were in their own HSD clustering group. Following those top two accessions were ‘DMC 04-61’ and ‘DMC 04-94’ (Table 3). Eleven PI accessions had a higher mean PPP compared to the highest averaging pod-yielding cultivar, ‘PL0014’, which produced 24.27 PPP (Table 3).

Comparatively, ‘Caprice’ serves as the standard cultivar in South Carolina, consistently delivering pods that meet the preferences of farmers and processing facilities. In the Tukey’s HSD of IPD, ‘Caprice’ ranked as the second highest cultivar in terms of mean PPP, averaging 34.72 PPP. Thirty-six SnAP accessions had higher mean PPP than ‘Caprice’ in IPD. In the Tukey’s HSD of HPD, ‘Caprice’ ranked fifth among cultivars, with an average of 20.07 PPP. Cultivars with higher mean than 20.07 PPP in

HPD were ‘PL0014’ (24.27 PPP), ‘SVG2106’ (23.1 PPP), ‘HM6401’ (22.2 PPP), and ‘CR-1849’ (20.57 PPP).

3.3 Genome-wide association study

A total of 28,978 SNPs were used for GWAS of the three traits evaluated. There was a total of 587 LD blocks with an average of 49 SNPs per LD block. A total of 30 significant associations were found across the 11 chromosomes (Table 4). By utilizing the Bayesian information content (BIC) file generated in GAPIT, we were able to determine that 0 principal components for each phenotypic trait in the MLM models was optimal, and this approach was applied to all other models. We found no significant chromosome associations in the single-locus MLM. There were 17 significant associations discovered using BLINK, and an additional 13 were found using FarmCPU. There was only a single SNP identified by both FarmCPU and Blink which was associated with PPP in the HPD on chromosome Pv11 at position 51,156,816 bp.

3.4 Days to flower SNPs

There were four significant SNPs found for DTF in the IPD at chromosomes Pv02, Pv07, Pv09, and Pv11 (Table 4). There were no significant SNPs associated with DTF in the HPD. Two of the four SNPs associated with DTF in the IPD had DNAJ - heat shock protein genes (Phvul.002G207300 and Phvul.011G210000) identified within their QTL interval (Table 5).

3.5 Pod production per plant SNPs

There were six significant SNPs found for PPP in IPD at chromosomes two at Pv03, two at Pv04, one at Pv05, and one at Pv08 (Table 4, Fig. 6). Of the thirty total SNPs found, S04_7512577 from PPP in IPD had the smallest p-value (Table 4). FarmCPU had a total of six SNPs for PPP in HPD, two of which were on chromosome Pv05. BLINK found two associations with PPP in the heat on chromosome Pv10 and Pv11 (Table 4, Fig. 9). Associations for PPP in HPD were found on Pv05, Pv07, Pv08, Pv10, Pv11 (Table 4, Fig. 9). SNP S08_58194729 had four different identification names (Phvul.008G227900, Phvul.008G228000, Phvul.008G228100, and Phvul.008G237000) all with the same HSP20 gene associated (Table 5). The other significant SNP from HPD with HSP40 and DNAJ superfamily protein was identified as Phvul.005G178800 (Table 5).

3.6 Weight of pods per plant SNPs

Pod weight per plant was associated with five significant SNPs in the IPD trial, located on chromosomes Pv02, Pv03, Pv06, Pv07, and Pv10 (Table 4). Two SNPs (S03_51082011 and S07_39436802) from IPD had HSP/HSF proteins (Phvul.003G276200 and Phvul.007G278200) within the QTL intervals (Table 5). KPP from HPD had seven significant associations on chromosomes Pv05, Pv06, Pv07, Pv09, and Pv10 (Table 4). SNP S09_16456444 was significant for its heat stable protein (HS1) at two start and stop intervals, identified as Phvul.009G108000 and Phvul.009G108100 (Table 5). Chromosome 9 seems to have a strong association with KPP and heat because SNP, S09_26058778, has heat related proteins (HSP90) as well. Lastly, Phvul.007G006200 contains a DNAJ and HSP40 cysteine-rich superfamily protein expressed in KPP from HPD (Table 5).

3.7 Allele dominance

Allele boxplots were created in R to visualize the allelic effect of significant SNPs from the GWAS. For DTF in IPD, S07_2193853 displayed the widest mean difference at 35 DTF for the "CC" genotype and 37 DTF for the "TT" genotype (Fig. 11). The overlapping significant SNP, S11_51156816, from BLINK and FarmCPU showed a difference in means ranging from 5 PPP ("TT") to 15 PPP ("CC") in HPD (Fig. 14). S05_19312453 has similar range in means with the lower amount of PPP with "TT" and the higher PPP with "CC" in HPD (Fig. 14). S07_183853 for KPP in HPD had a difference of 0.03 KPP and this SNP was identified with a heat shock protein gene. (Table 5, Fig. 15).

Discussion

The rise in temperatures across the globe is inevitable, therefore research focused on heat stress and heat tolerance is becoming more critical to our future food security. This is the first GWAS using the SnAP to evaluate flowering time and pod production of determinate snap bean types under heat stress conditions.

4.1 Trait discussion

The results of our field experiments confirmed that heat stress negatively affects snap bean yields. Correlations between DTF and PPP/KPP indicated that in HPD the earlier flowering on average led to higher pod set. Plants requiring more than 60 days to reach maturity often resulted in a below average pod yield. The environment and reproduction stages of flowering can positively or negatively affect a plant. For example, early flowering in the HPD may have avoided an extended period of heat and/or escape pathogen attacks which could have negatively affected seed and pod production (Raggi et al. 2019).

There was a correlation between PPP and KPP. Specifically, plants with higher PPP tend to yield pods of greater weight (KPP) overall, in comparison to those with lower pod production. However, the accession with the highest pod count does not necessarily equate to the highest pod weight. This can be attributed to the variations in pod structure, including sieve size, water and seed content, which significantly influence the overall weight of pods per plant.

4.2 Genetic discussion

Vargas et al. (2021) found QTLs for the number of pods per plant in a dry bean population at Pv01, Pv04, and Pv08 under heat-stressed conditions. Our results also identified significant SNPs at chromosomes Pv04 and Pv08 (S04_34273966, S08_4198189) in PPP from HPD (Table 4). Unlike Vargas et al. (2021), who reported significant associations with DTF under heat stress, this GWAS did not detect any significant associations for DTF in HPD. Oladzad et al. (2019) used a Bean Abiotic Stress Evaluation (BASE) approach with Middle American, Andean, and tepary bean genotypes, which found SNPs at Pv03, Pv08, and Pv11 for yield under heat stress (Oladzad et al. 2019). Similarly, our GWAS of ~300 lines with both Middle American and Andean origins found pod production SNPs at Pv08 and Pv11 under heat (Table 4). Another GWAS using the Andean Diversity Panel (ADP) (n=237) found SNPs for pod number at Pv05 and Pv07 and yield per plant associated with Pv08 and Pv09 (Kamfwa et al. 2015). Although they did not evaluate heat stress, we did see overlap in our yield traits (PPP, KPP) and chromosome associations for IPD and HPD. Pod production is a promising trait to include for selection in breeding programs for its economic importance and moderately high heritability. A bi-parental heat tolerance study also reported high heritability for pod count (0.74) (Rainey and Griffiths 2005).

4.3 Heat shock proteins

A total of 36,342 genes were identified in the intervals of 500,000 mb up and down of the significant SNPs discovered in GAPIT. Of the thirty significant SNPs, thirteen co-located with genes encoding heat shock proteins. Heat shock transcription factors (Hsf) regulate plant defense system against biotic and abiotic stress (Zhang et al. 2022). They can activate the heat shock proteins (HSP) which promote refolding, assembly, distribution, and decomposition of damaged proteins (Zhang et al. 2022). The only significant SNP near heat shock related proteins from the pod production trait in heat-stressed condition was S05_40579080 (Table 4, Table 5).

A heat tolerance study of genome-environment association in dry beans found similar gene proteins, HSP40 associated with Pv02, Pv03, and Pv06 (López-Hernández and Cortés et al. 2019). Although we also found heat shock proteins on Pv02 (DTF) and Pv03 (KPP), they were with traits in the ideal environment (Table 5). A study using common bean further examined heat shock transcription factors (PvHsfs) expression from the phytozome database and the analysis at the sprout stage through different tissues revealed that PvHsfs had tissue-specific expression (Zhang et al. 2022). High gene expression levels of PvHsfs in the common bean under heat that were highlighted in pods were identified as PvHsf05, PvHsf21, and PvHsf22 (Zhang et al. 2022).

4.4 Future breeding objectives

Additional phenotypic traits to incorporate into a snap bean breeding program to enhance marketability include pollen viability, seed fill, curvature, and moisture content. Subsequently, the most promising lines will undergo a refined selection process to identify the optimal parent lines. To increase selectivity and success rate, a Multi-parent Advanced Generation Inter-Cross (MAGIC) population could be developed in future breeding programs (Diaz et al. 2020). MAGIC populations have been used successfully for *Arabidopsis thaliana*, wheat, rice, chickpea, and other crops (Huang et al. 2015).

Conclusion

Overall, we can conclude that snaps beans are susceptible to the impacts of increasing temperatures, which is inevitable with the current climate outlook (Langstroff et al. 2022). This research, alongside numerous other studies, are funded to better comprehend the vulnerability and sensitivity plants have to abiotic stresses (Gallegos et al. 2020). This collective knowledge serves as a cornerstone in preparing and proactively addressing future challenges. Developing cultivars with yield stability under unfavorable environmental conditions (heat/drought) can minimize crop loss and prevent complete crop failure (Boyles et al. 2019). The nearly 50% decrease in pod production observed under heat stress over two years of field trials suggests a crucial need to continue research and breeding objectives in snap beans. There is a promising future for breeding heat tolerant snap beans. This strategic approach, focused on identifying and utilizing specific markers associated with heat tolerance, has the potential to extend the growing season, broaden production regions, and increase resilience against temperature fluctuations.

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APPENDICES

List of Tables

Table 1. Summary statistics for each phenotypic trait in ideal and heat planting environments.

Statistic	PPP [a] _ideal	KPP [b] _ideal	DTF [c] _ideal	PPP_heat	KPP_heat	DTF_heat
Mean	28.68	0.14	35.19	14.41	0.05	33.51
Min	1.4	0	31	0	0	28
Max	71.6	0.42	43	53	0.25	47
St.dev [d]	10.53	0.06	1.66	10.08	0.04	3.28
H^2 [e]	0.67	0.64	0.89	0.57	0.62	0.71
V_p [f]	110.81	0	2.76	101.63	0	10.77
Correlation of year (P) [g]	1.61E-15	3.47E-13	< 2.2e-16	1.20E-14	< 2.2e-16	< 2.2e-16
Correlation of year (*r*) [h]	0.50	0.46	0.73	0.48	0.54	0.55

a. PPP = pods per plant
b. KPP = kilograms per plant
c. DTF = days to flower
d. St.dev is the standard deviation
e. Broad-sense hertiability
f. Phenotypic variance
g. Correlation of between two year as measured p-value (p)
h. Correlation of between two year as measured by Pearson's correlation coefficient *(r)*

Table 2. Highest pod producing PI accessions in comparison to cultivars for the ideal trial.

Name	Type	PPP [a] ideal means	Group [b]
Baby_Bop	PI accession	48.93	a
Dandy	PI accession	47.13	ab
Flavor_Sweet	PI accession	43.37	abc
Minuette	PI accession	43.03	a-d
Slenderwax	PI accession	42.36	a-e
Sea_biscuit	PI accession	41.3	a-f
Dynasty	PI accession	41	a-g
Slenderpack	PI accession	40.2	a-h
Brio	PI accession	39.77	a-h
Ovation	PI accession	39.5	a-i

Smilo	PI accession	39.16	a-j
Win	PI accession	39.13	a-j
Mirada	PI accession	38.57	a-j
Lynx	PI accession	38.36	a-j
Impact	PI accession	37.5	a-j
Tanta	PI accession	37.2	a-j
Legion	PI accession	37	a-j
Epoch	PI accession	36.97	a-j
Sunrae	PI accession	36.97	a-j
Acclaim	PI accession	36.77	a-j
Kylian	PI accession	36.43	a-k
Rocdor	PI accession	36.03	a-l
Benton	PI accession	36	a-l
Festina	PI accession	35.93	a-l
Thoroughbred	PI accession	35.93	a-l
HM6401	cultivar	35.87	a-l
Flevoro	PI accession	35.8	a-l
Tema	PI accession	35.63	a-l

a. PPP represents pods per plant.

b. Group is determined by the difference of pods between each accession (Tukey's HSD).

Table 3. Highest pod producing PI accessions in comparison to cultivars for the heat trial.

Name	**Type**	**PPP [a] heat means**	**Group [b]**
Flavor_Sweet	PI accession	31.17	a
Dandy	PI accession	29.7	ab
DMC_04-61	PI accession	28.97	abc
DMC_04-94	PI accession	28.68	a-d
Stallion	PI accession	26.73	a-e
Blue_Knight	PI accession	26.3	a-f
Smilo	PI accession	25.36	a-g
Rainier	PI accession	25.07	a-g
Brio	PI accession	25.01	a-h
Molly	PI accession	24.87	a-h
Oregon_1604M	PI accession	24.84	a-i
PL0014	cultivar	24.27	a-i
Impact	PI accession	24.17	a-i

a. PPP represents pods per plant.

b. Group is determined by the difference of pods between each accession (Tukey's HSD).

Table 4. Significant SNPs for each trait, GAPIT model, environment, chromosome, and position, and significance levels.

Trait	Model	Envir.	SNP	Chr.	Position	P-value	MAF [a]	FDR [b] <0.01
DTF	BLINK	ideal	S02_37126080	2	37126080	2.31E-12	0.26	6.61E-08
DTF	BLINK	ideal	S07_2193853	7	2193853	1.91E-07	0.01	1.36E-03
DTF	BLINK	ideal	S09_2721726	9	2721726	1.34E-08	0.09	1.27E-04
DTF	BLINK	ideal	S11_52675259	11	52675259	1.12E-11	0.1	1.60E-07
KPP	BLINK	ideal	S02_42035839	2	42035839	1.04E-07	0.46	7.43E-04
KPP	BLINK	ideal	S03_51082011	3	51082011	9.94E-08	0.18	7.43E-04
KPP	BLINK	ideal	S07_39436802	7	39436802	8.53E-08	0.11	7.43E-04
KPP	BLINK	ideal	S10_44213690	10	44213690	7.16E-09	0.44	2.05E-04
KPP	FarmCPU	ideal	S06_29130696	6	29130696	1.55E-08	0.12	4.43E-04
PPP	BLINK	ideal	S03_2541311	3	2541311	1.08E-06	0.07	5.15E-03
PPP	BLINK	ideal	S03_46891445	3	46891445	1.48E-08	0.1	1.06E-04
PPP	BLINK	ideal	S04_22201577	4	22201577	2.27E-09	0.13	3.24E-05
PPP	BLINK	ideal	S04_7512577	4	7512577	1.41E-14	0.27	4.03E-10
PPP	BLINK	ideal	S05_37923469	5	37923469	5.22E-07	0.13	2.98E-03
PPP	BLINK	ideal	S08_58194729	8	58194729	9.17E-09	0.13	8.73E-05
KPP	BLINK	heat	S06_19917667	6	19917667	9.86E-10	0.05	2.82E-05
KPP	FarmCPU	heat	S05_2078141	5	2078141	1.62E-06	0.44	8.43E-03
KPP	FarmCPU	heat	S05_2895787	5	2895787	3.25E-07	0.47	3.10E-03
KPP	FarmCPU	heat	S07_183853	7	183853	7.81E-07	0.05	5.58E-03
KPP	FarmCPU	heat	S09_16456444	9	16456444	6.81E-10	0.4	1.95E-05
KPP	FarmCPU	heat	S09_26058778	9	26058778	1.77E-06	0.15	8.43E-03
KPP	FarmCPU	heat	S10_44211281	10	44211281	1.48E-09	0.43	2.12E-05
PPP	BLINK	heat	S10_4906741	10	4906741	5.31E-07	0.35	7.58E-03
PPP	BLINK	heat	S11_51156816	11	51156816	1.21E-07	0.02	3.45E-03
PPP	FarmCPU	heat	S04_34273966	4	34273966	1.66E-07	0.08	1.19E-03
PPP	FarmCPU	heat	S05_19312453	5	19312453	2.67E-08	0.06	3.81E-04
PPP	FarmCPU	heat	S05_40579080	5	40579080	3.45E-07	0.08	1.97E-03
PPP	FarmCPU	heat	S07_32673725	7	32673725	9.07E-09	0.23	2.59E-04
PPP	FarmCPU	heat	S08_4198189	8	4198189	8.49E-08	0.41	8.09E-04
PPP	FarmCPU	heat	S11_51156816	11	51156816	5.81E-07	0.02	2.77E-03

a. MAF is abbreviated for minor allele frequency.

b. FDR is the false discover rate at less than 0.01.

Table 5. Prioritized candidate genes based upon functional annotation.

SNP	Chr.	Name	Start	Stop	Protein Defined
S02_37126080	Chr02	Phvul.002G207300	37387663	37388398	DNAJ-like 20
S03_51082011	Chr03	Phvul.003G276200	51372196	51374180	HSP20-like chaperones superfamily protein
S05_40579080	Chr05	Phvul.005G178800	40425947	40433162	Molecular chaperone Hsp40/DnaJ family protein
S07_183853	Chr07	Phvul.007G006200	462361	465223	DnaJ/Hsp40 cysteine-rich domain superfamily protein
S07_39436802	Chr07	Phvul.007G278200	39805254	39807144	Heat shock transcription factor A6B (HSFA6B)
S08_58194729	Chr08	Phvul.008G227900	57808676	57809396	HSP20-like chaperones superfamily protein
S08_58194729	Chr08	Phvul.008G228000	57812230	57813077	HSP20-like chaperones superfamily protein
S08_58194729	Chr08	Phvul.008G228100	57815075	57815554	HSP20-like chaperones superfamily protein
S08_58194729	Chr08	Phvul.008G237000	58548465	58550096	HSP20-like chaperones superfamily protein
S09_16456444	Chr09	Phvul.009G108000	16740512	16742408	Heat stable protein 1 (HS1)
S09_16456444	Chr09	Phvul.009G108100	16762151	16763330	Heat stable protein 1 (HS1)
S09_26058778	Chr09	Phvul.009G176500	26215676	26237916	Histidine kinase-; DNA gyrase B-; and HSP90-like ATPase family protein
S11_52675259	Chr11	Phvul.011G210000	52747036	52754695	DNAJ heat shock N-terminal domain-containing protein

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Figure 1. Histogram of accession means of pods per plant under ideal and heat-stressed planting dates across years. The dotted line indicates the snap bean panel mean (across accessions).

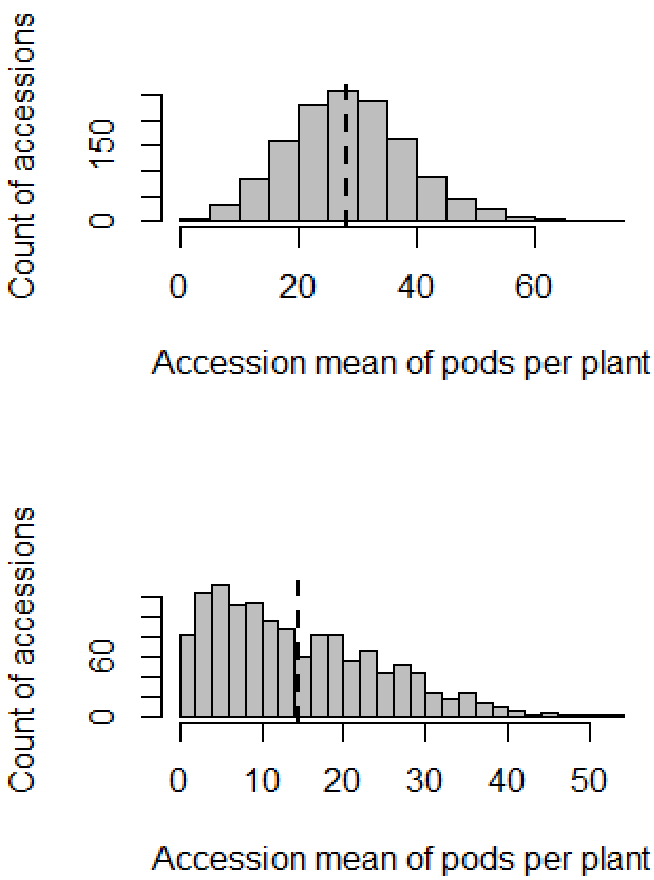


Figure 2. Weather data from the **ideal** planting treatment for year one and two. Red is the max (day) Celcius temperature and blue is the min (night) Celcius temperature. The dotted line is a threshold of 27°Celsius (80°F) for day-time and 21°Celsius (70°F) for night-time.

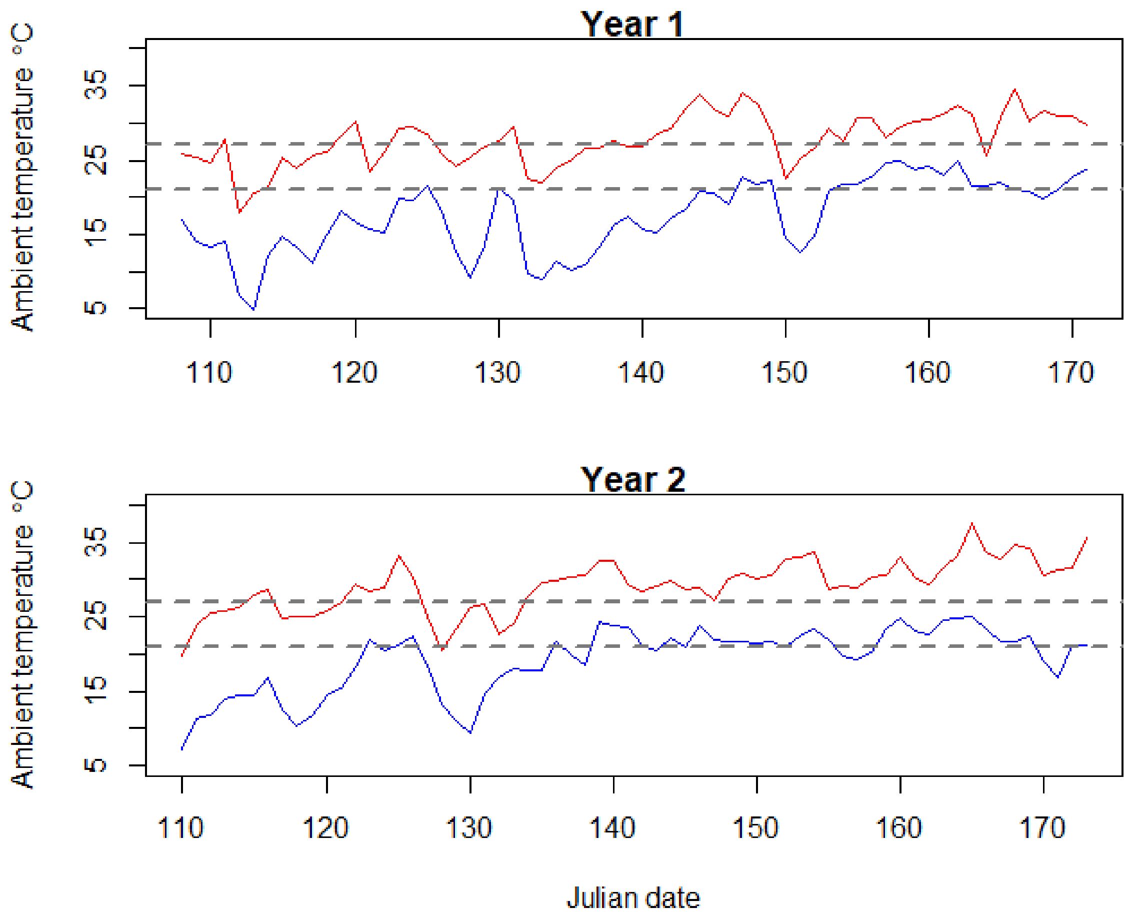


Figure 3. Weather data from the **heat** planting treatment for year one and two. The red line is max (day) temperatures in Celsius and the blue line is the min (night) temperatures. Many points are above the dotted line, showing heat stress was induced during the day, and at night.

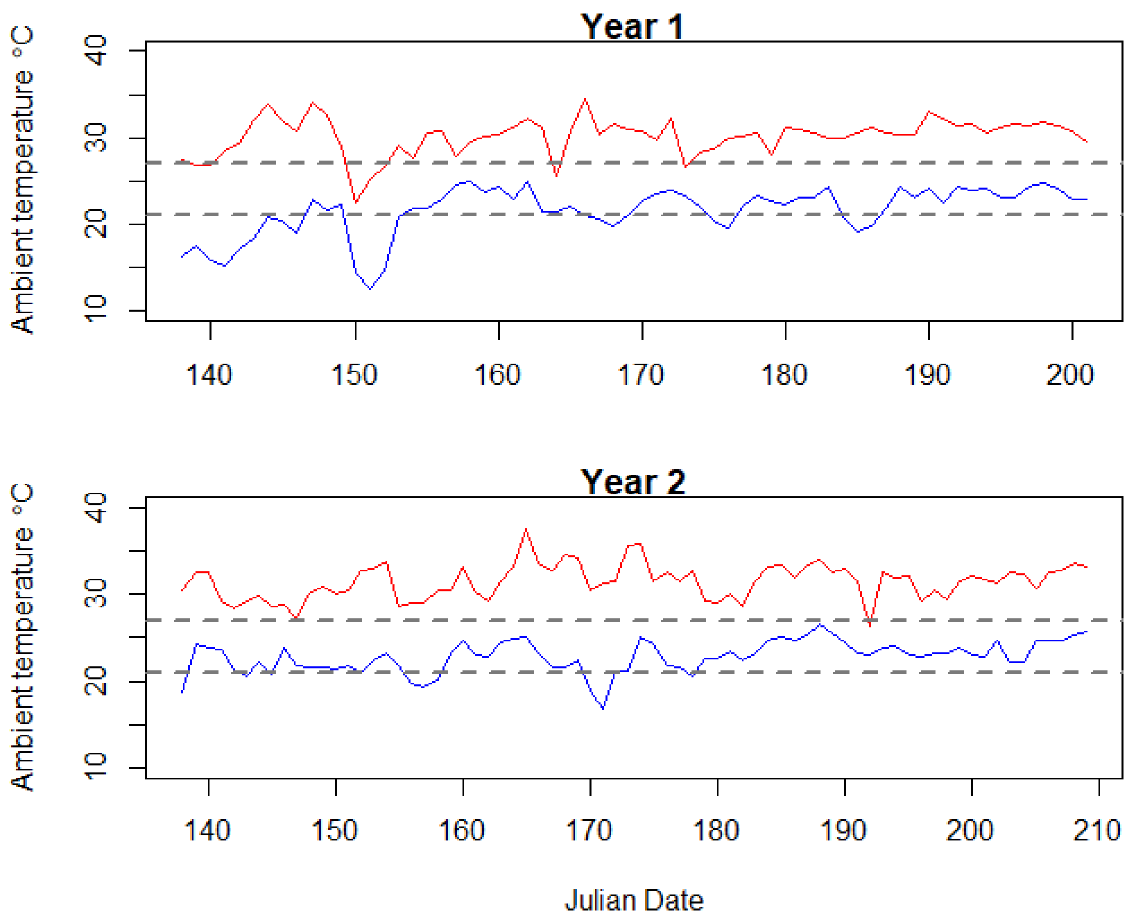


Figure 4. Weather data of combined ideal and heat of both years for closer comparison. On average ideal will have data points within the dotted lines, whereas the heat data is seen mostly above the dotted lines. Data begins to overlap at Julian day 140-170.

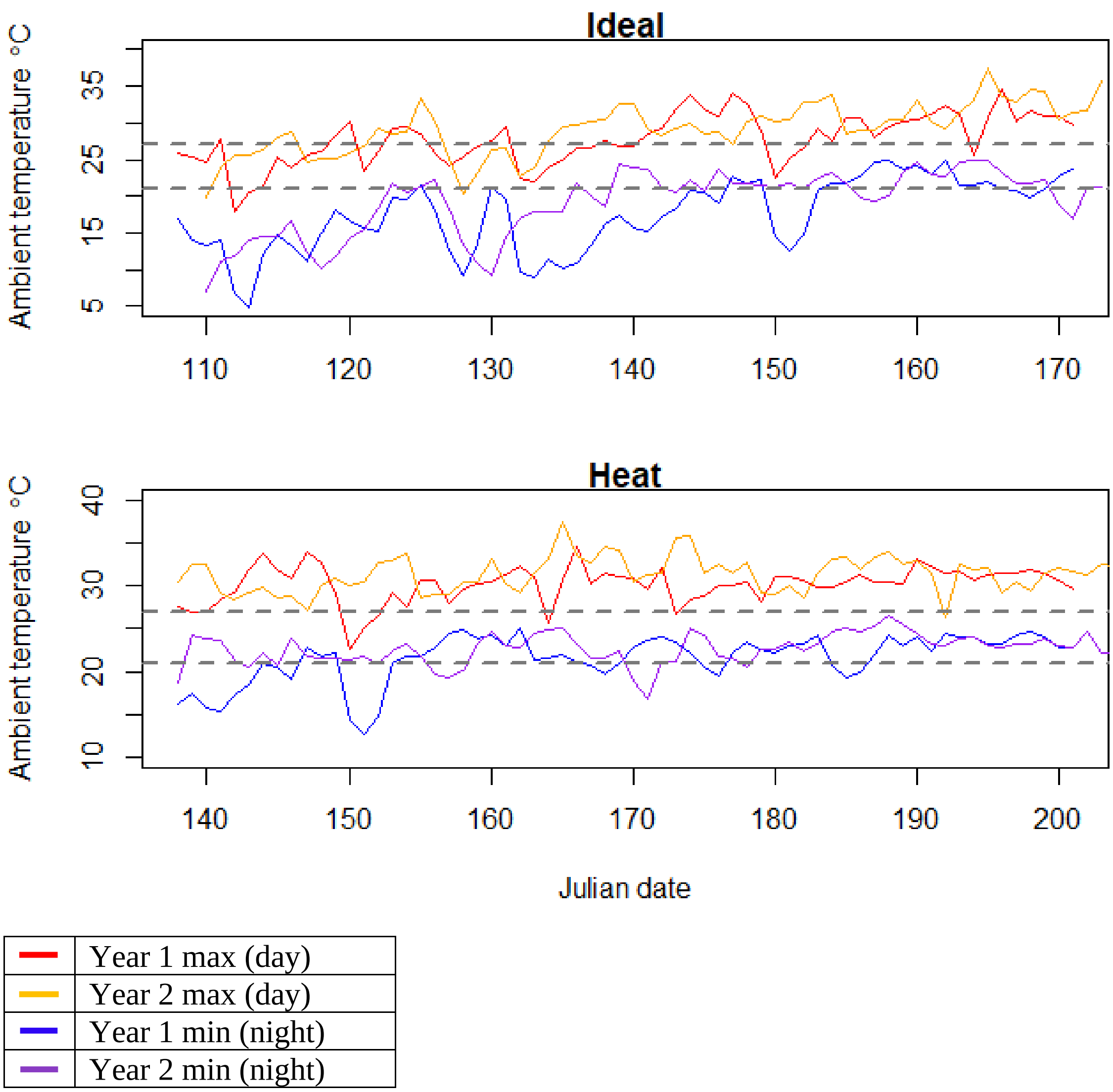


Figure 5. Manhattan and qq plots for the days to flower in ideal planting (dtfi) across years. Genome wide significance thresholds are indicated by the horizontal line.

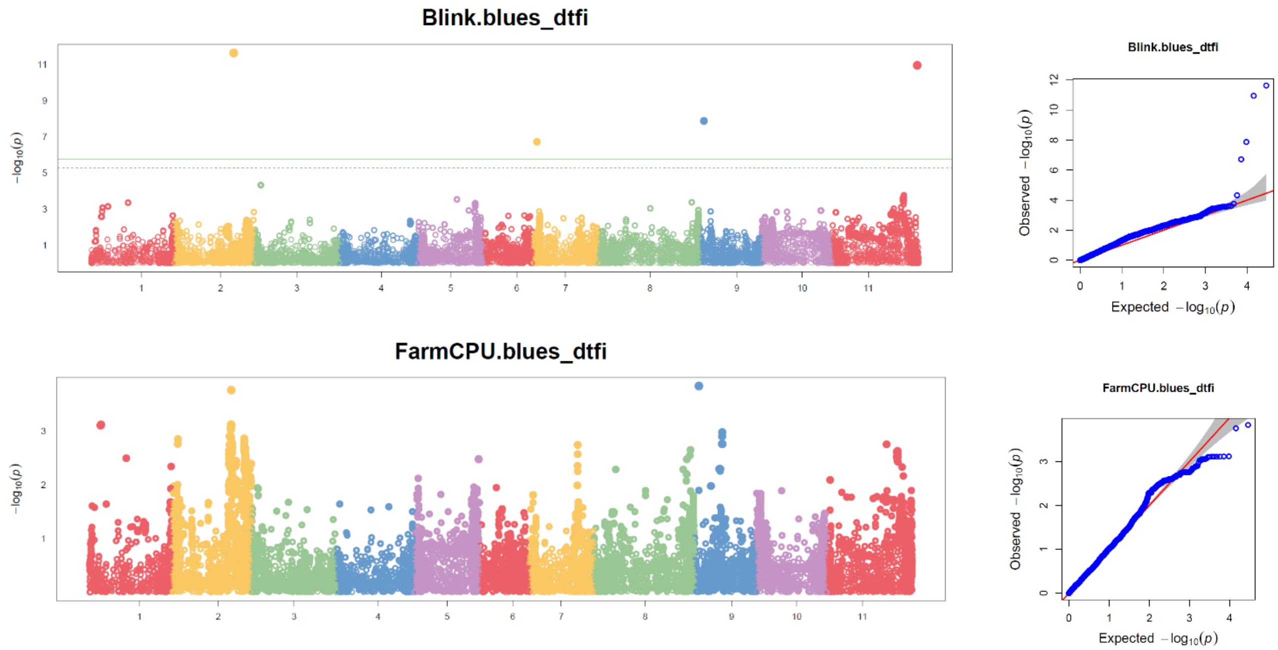


Figure 6. Manhattan and qq plots for pods per plant in ideal (pppi) planting date of combined years. Genome wide significance thresholds are indicated by the horizontal line.

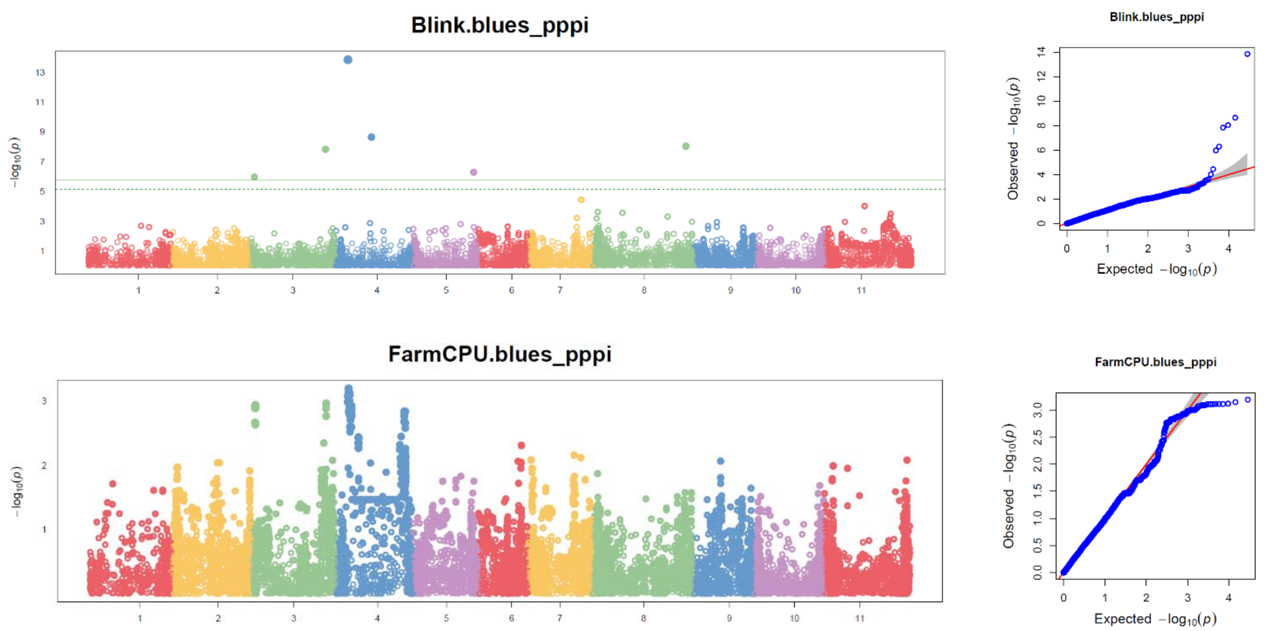


Figure 7. Manhattan and qq plots for kilograms per plant in ideal (kppi) planting dates of combined years. Genome wide significance thresholds are indicated by the horizontal line.

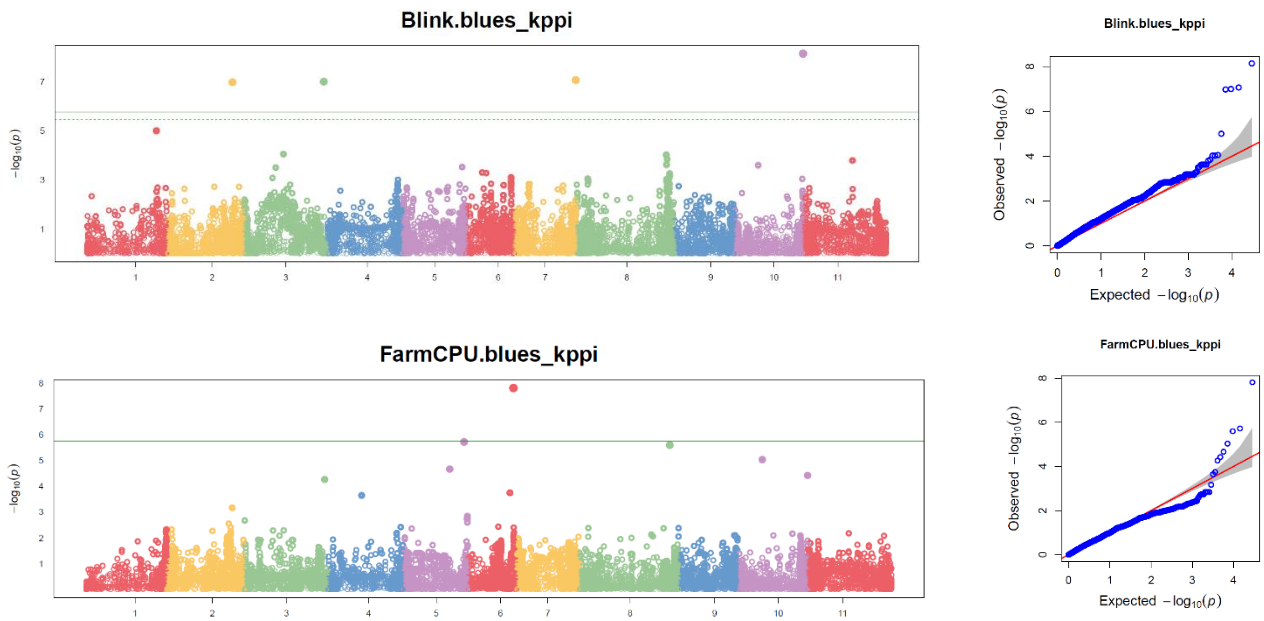


Figure 8. Manhattan and qq plots for days to flower in heat (dtfh) planting date from the blues of both years. Neither model has signficant assocations.

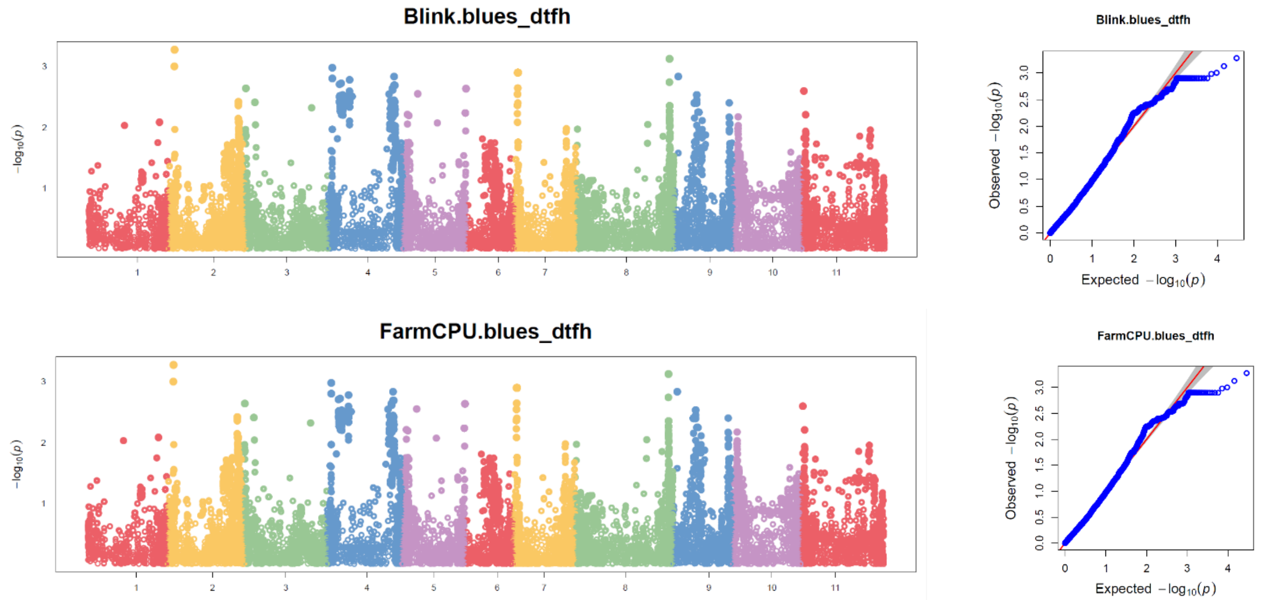


Figure 9. Manhattan and qq plots for pods per plant in heat (ppph). Genome wide significance thresholds are indicated by the horizontal line.

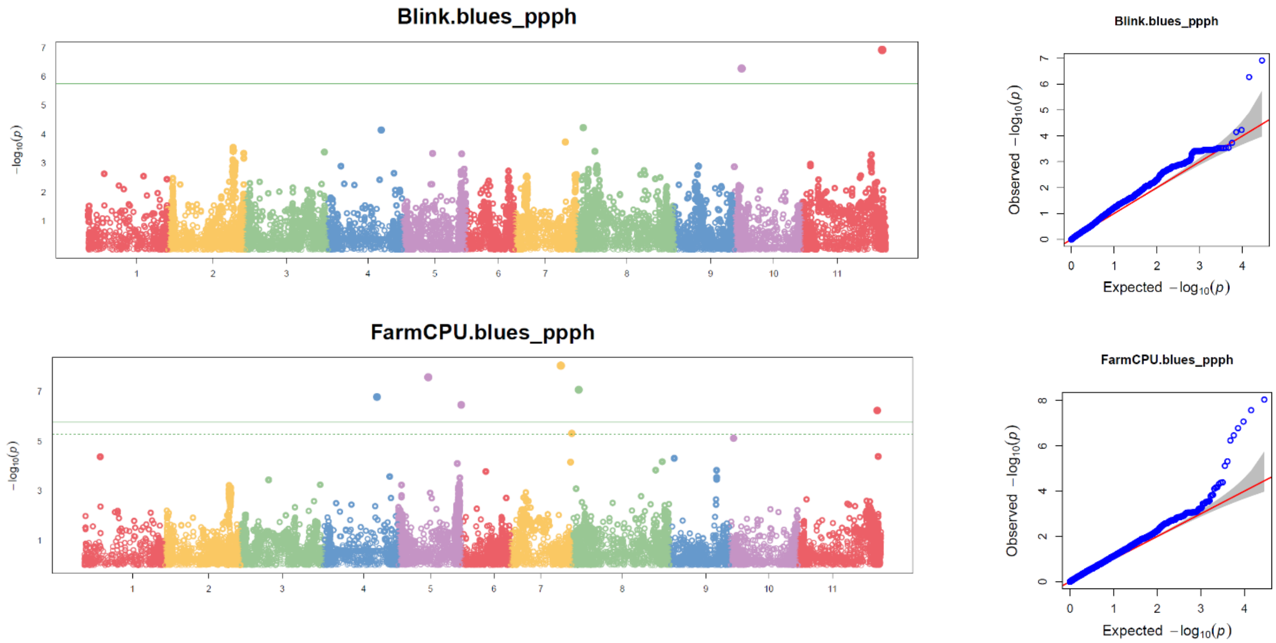


Figure 10. Manhattan plots for kilograms per plant in heat (kpph). Genome wide significance thresholds are indicated by the horizontal line.

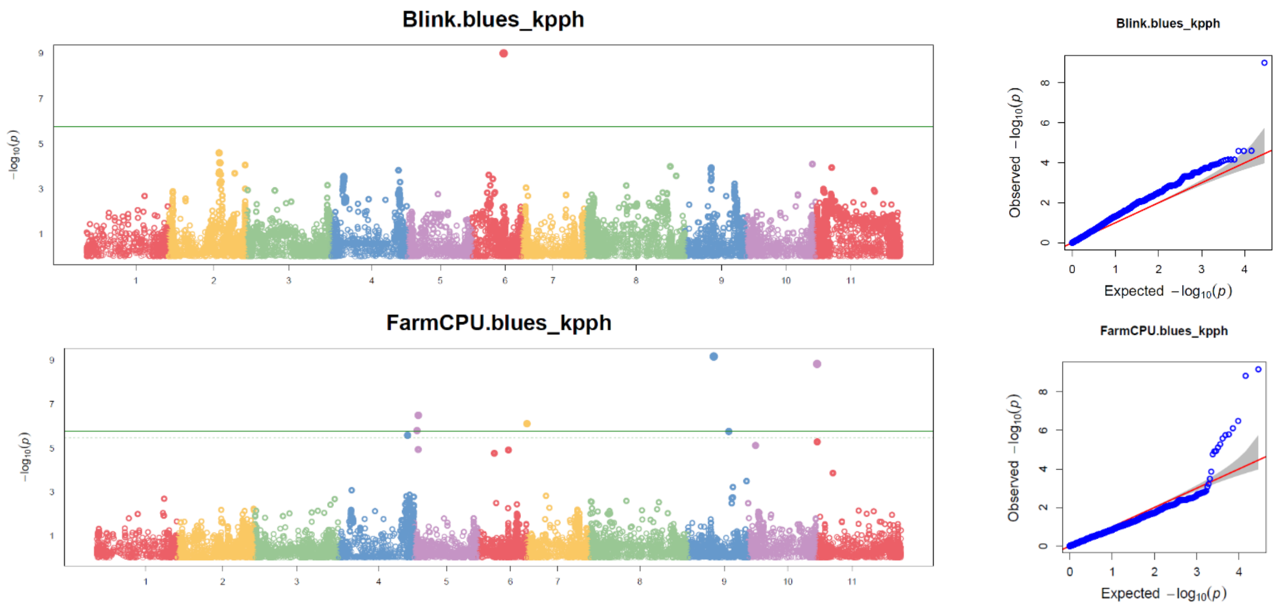


Figure 11. Box plots of significant SNP genotypes in the DTF ideal planting.

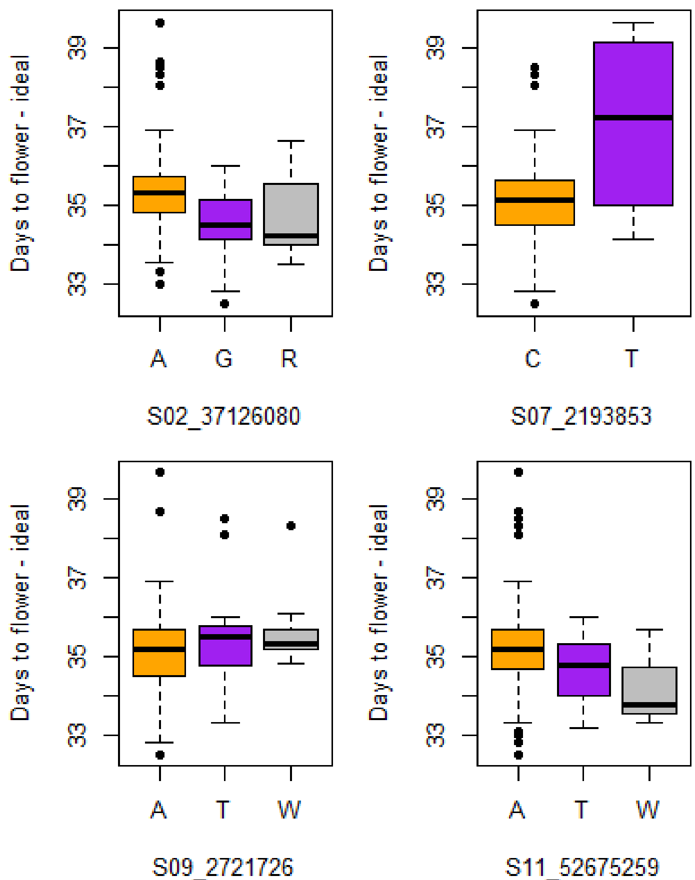


Figure 12. Box plots of genotypes from significant SNPs in the PPP ideal planting.

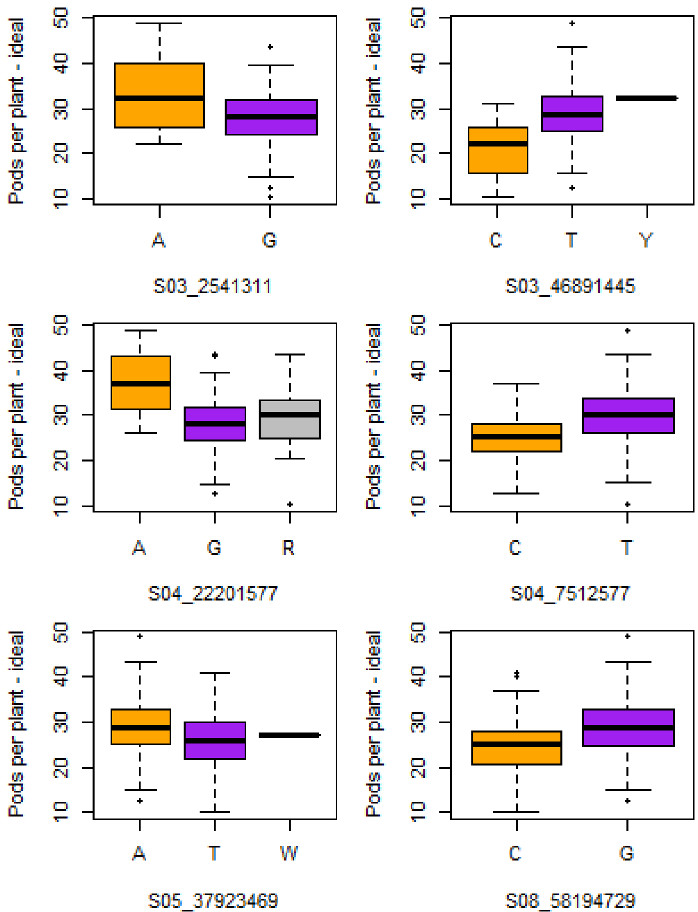


Figure 13. Box plots of genotypes from significant SNPs in the KPP ideal planting.

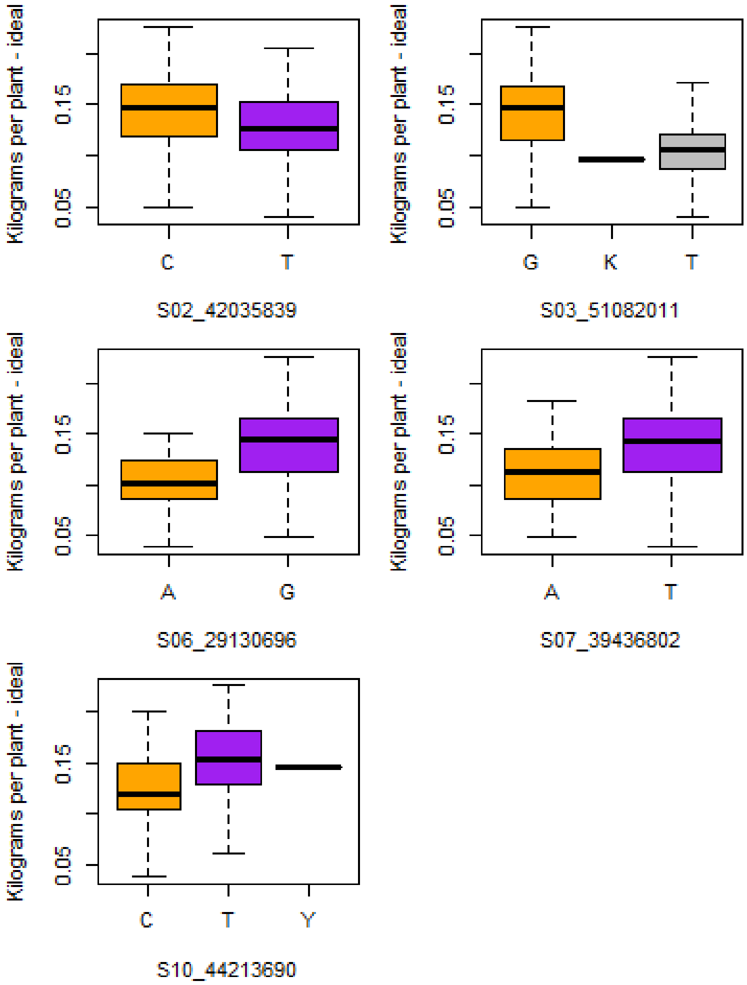


Figure 14. Box plots of genotypes from significant SNPs in the PPP heat planting.

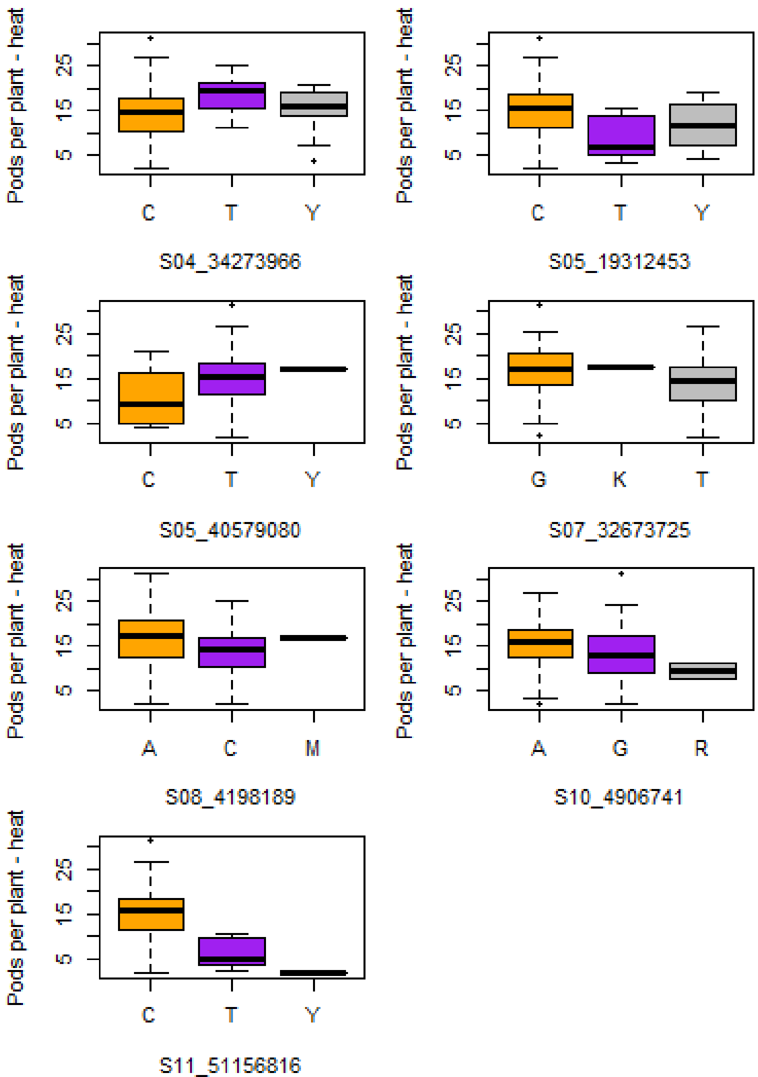
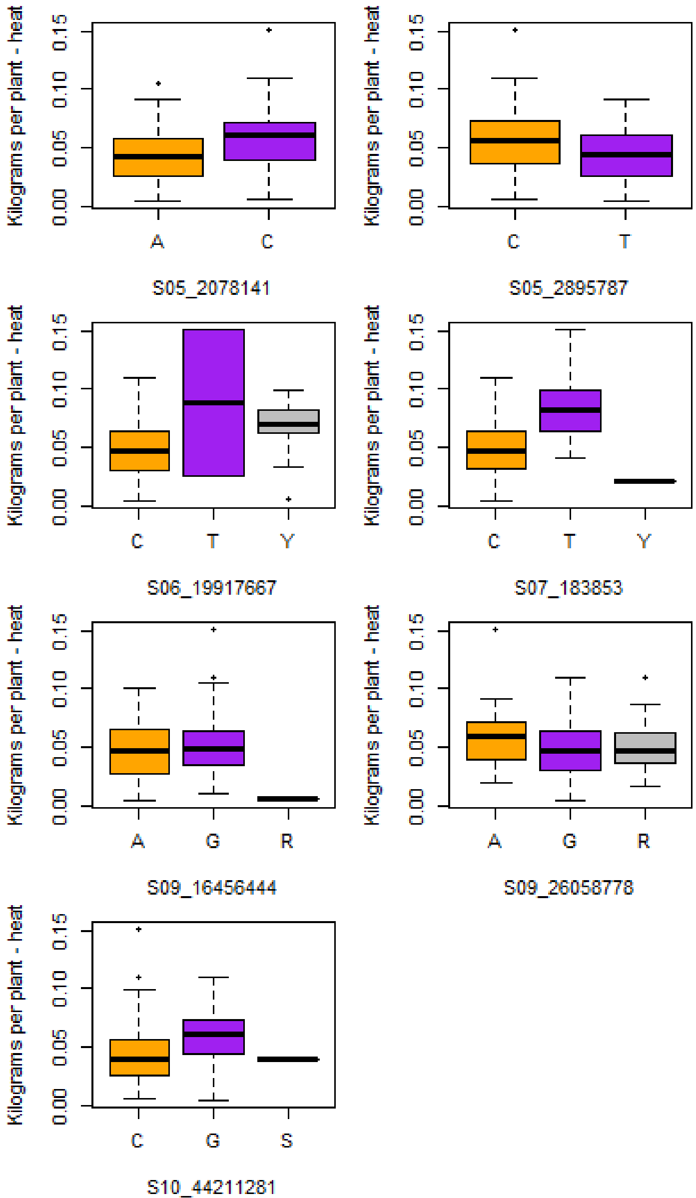


Figure 15. Box plots of genotypes from significant SNPs in the KPP ideal planting



Supplementary Table

Supp. Table 1. Collection information for the accessions used in the genome-wide association study.

Cultivar	Source	Bean CAP. Entry	PI#	Type	Origin	PVP.Date .of.Issue.
Acclaim	BeanCAP	1	PI 550420	processing	Asgrow Seed Company	1992
Angers	BeanCAP	2	NA	fresh market	Seminis Vegetable Seeds Inc.	NA
Astun	BeanCAP	3	PI 632998	romano	Syngenta	2003
Balsas	BeanCAP	4	NA	proccessing (whole)	Syngenta Seeds Inc.	NA
Banga	BeanCAP	5	PI 651600	proccessing (whole)	Seminis Vegetable Seeds Inc.	2008
BBL_156	BeanCAP	6	PI 550403	processing	Rogers NK Seed Co.	1992
BBL_274	BeanCAP	7	PI 549837	processing	Asgrow Seed Company	NA
Benchmark	BeanCAP	8	PI 596570	fresh market	Novartis Seeds inc.	2001
Benton	BeanCAP	9	PI 550043	proccessing	Gallatin Valley Seed Co.	1986
Black_Valentine	BeanCAP	10	PI 152456	fresh market	Peter Henderson and Company	NA
Bogota	BeanCAP	11	NA	romano	Seminis Vegetable Seeds Inc.	NA
Booster	BeanCAP	12	NA	processing (whole)	Syngenta Seeds Inc.	withdrawn
Brio	BeanCAP	13	PI 550421	proccessing	Asgrow Seed Company	1992
Brittle_Wax	BeanCAP	14	PI 549541	dual	Johnson & Stokes orig. Calvin Keeney	NA
Bronco	BeanCAP	15	PI 550281	fresh market	Asgrow Seed Company	1990
Cadillac	BeanCAP	16	PI 635099	processing (whole)	Seminis Vegetable Seeds Inc.	2007
Calgreen	BeanCAP	17	PI 538772	KY flat	Rogers NK Seed Co.	1992
Carlo	BeanCAP	18	PI 594389	dual	Seminis Vegetable Seeds Inc.	2001

Carson	BeanCAP	19	PI 634346	wax	Syngenta Seeds Inc.	2007
Castano	BeanCAP	20	PI 612143	processing	Syngenta Seeds Inc.	2002
Catania	BeanCAP	21	NA	proccessing (whole)	Seminis Vegetable Seeds Inc.	NA
Celtic	BeanCAP	22	PI 564739	proccessing (whole)	Rogers NK Seed Co.	1993
Charon	BeanCAP	23	PI 618599	fresh market	Syngenta Seeds Inc.	2002
Cherokee	BeanCAP	24	PI 549543	fresh market	Clemson	NA
Coloma	BeanCAP	25	PI 549954	processing	Rogers Brothers Seed Company	NA
Contender	BeanCAP	26	PI 549526	fresh market	USDA Veg Breeding Lab.	NA
Cyclone	BeanCAP	27	PI 599321	KY flat	Seminis Vegetable Seeds Inc.	2001
Dandy	BeanCAP	28	PI 550037	proccessing (whole)	Rogers Brothers Seed Company	1983
Derby	BeanCAP	29	PI 550150	processing	Ferry-Morse Seed Company	1992
Doral	BeanCAP	30	PI 628970	processing (whole)	Syngenta Seeds Inc.	2002
Dubbele_Witte	BeanCAP	31	PI 598994	fresh market	IVT	NA
Dusky	BeanCAP	32	PI 632370	fresh market	Syngenta Seeds Inc.	2003
Eagle	BeanCAP	33	PI 549914	dual	Asgrow Seed Company	1974
Ebro	BeanCAP	34	PI 615080	romano	Seminis Vegetable Seeds Inc.	2002
Embassy	BeanCAP	35	PI 639523	processing	Syngenta Seeds Inc.	2007
Envy	BeanCAP	36	PI 561051	processing	Ferry-Morse Seed Company	1993
Espada	BeanCAP	37	PI 537106	processing	Harris Moran Seed Company	1992
Esquire	BeanCAP	38	PI 619196	processing	Syngenta Seeds Inc.	2002
EZ_Pick	BeanCAP	39	PI 550255	proccessing	NPI AgService Corporation	1987
Ferrari	BeanCAP	40	NA	fresh market	Bakker Brothers	abandoned
Festina	BeanCAP	41	PI 606782	dual	Seminis Vegetable Seeds Inc.	2001

Flavio	BeanCAP	42	NA	fresh market	Seminis Vegetable Seeds Inc.	NA
Flavor_Sweet	BeanCAP	43	NA	proccessing (whole)	Harris Moran Seed Company	NA
FR_266	BeanCAP	45	NA	proccessing	Seminis Vegetable Seeds Inc.	NA
Fury	BeanCAP	46	PI 612597	proccessing	Seminis Vegetable Seeds Inc.	2002
Gallatin_50	BeanCAP	47	PI 549664	proccessing	Gallatin Valley Seed Company	NA
Galveston	BeanCAP	48	PI 656654	proccessing	Syngenta Seeds Inc.	2009
Gina	BeanCAP	49	PI 549915	romano	Asgrow Seed Company	1974
Gold_Mine	BeanCAP	50	PI 546491	wax - dual	Asgrow Seed Company	1992
Goldrush	BeanCAP	51	PI 549977	wax	Asgrow Seed Company	1977
Green_Arrow	BeanCAP	52	NA	NA	Seminis Vegetable Seeds Inc.	NA
Grenoble	BeanCAP	53	NA	dual	Seminis Vegetable Seeds Inc.	NA
Hayden	BeanCAP	54	PI 641960	processing	Syngenta Seeds Inc.	2007
Hercules	BeanCAP	55	PI 612168	processing	Seminis Vegetable Seeds Inc.	2002
Hialeah	BeanCAP	56	PI 550151	fresh market	Ferry-Morse Seed Company	1992
Hystyle	BeanCAP	57	PI 550288	proccessing	Harris Moran Seed Company	1988
Idaho_Refugee	BeanCAP	58	PI 549551	processing	University of Idaho	NA
Igloo	BeanCAP	59	PI 596753	processing	Hague-Igloo Vegetable Seeds	1999
Impact	BeanCAP	60	PI 565115	wax - processing	Asgrow Seed Company	1994
Jade	BeanCAP	61	PI 559394	fresh market	Rogers NK Seed Co.	1992
Koala	BeanCAP	62	NA	fresh market	Seminis Vegetable Seeds Inc.	NA
Kylian	BeanCAP	63	NA	fresh market	Seminis Vegetable Seeds Inc.	NA
Labrador	BeanCAP	64	PI 550118	dual	Asgrow Seed Company	1986
Landmark	BeanCAP	65	NA	fresh market	Musser Seed Co.	abandoned

Landreths_Stringless	BeanCAP	66	NA	fresh market	NA	NA
Magnum	BeanCAP	67	PI 550424	KY flat	Asgrow Seed Company	1992
Masai	BeanCAP	68	PI 566907	processing (whole)	Rogers Seed Company	1997
Matador	BeanCAP	69	PI 570648	processing	Asgrow Seed Company	1996
Medinah	BeanCAP	70	PI 608758	processing-whole	Novartis Seeds Inc.	2001
Mercury	BeanCAP	71	PI 612144	fresh market	Syngenta Seeds Inc.	2002
Minuette	BeanCAP	72	PI 583748	processing	Harris Moran Seed Co.	1999
Navarro	BeanCAP	73	PI 634725	romano	Harris Moran Seed Company	2007
Nicelo	BeanCAP	74	PI 594390	proccessing	Seminis Vegetable Seeds Inc.	2001
Nomad	BeanCAP	75	NA	processing (whole)	Seminis Vegetable Seeds Inc.	NA
Normandie	BeanCAP	76	NA	processing (whole)	Harris Moran Seed Company	NA
NY6020-5	BeanCAP	77	NA	Breeding line	Cornell University	NA
Opus	BeanCAP	78	PI 538026	fresh market	Asgrow Seed Company	1992
Oregon_1604M	BeanCAP	79	NA	processing	Oregon State University	NA
Oregon_2065	BeanCAP	80	NA	processing	Oregon State University	NA
Oregon_5402	BeanCAP	81	NA	processing	Oregon State University	NA
Oregon_91G	BeanCAP	82	NA	processing	Oregon State University	NA
Oregon_5630	BeanCAP	83	NA	processing	Oregon State University	NA
Palati	BeanCAP	84	PI 619195	fresh market	Syngenta Seeds Inc.	2002
Paloma	BeanCAP	85	NA	processing (whole)	Nunhems Seed Coporation	NA
Panama	BeanCAP	86	NA	fresh market	Seminis Vegetable Seeds Inc.	NA
Paulista	BeanCAP	87	NA	NA	Syngenta Seeds Inc.	NA
Pix	BeanCAP	88	PI 599322	processing-whole	Seminis Vegetable Seeds Inc.	2001

Polder	BeanCAP	89	PI 603217	processing-whole	Vilmorin S.A.	2002
Teresa_(Pretoria)	BeanCAP	90	NA	fresh market	Seminis Vegetable Seeds Inc.	NA
Profit	BeanCAP	91	NA	fresh market	Seminis Vegetable Seeds Inc.	NA
Prosperity	BeanCAP	92	PI 576167	fresh market	Harris Moran Seed Company	1995
Provider	BeanCAP	93	NA	fresh market	USDA Veg Breeding Lab.	NA
Redon	BeanCAP	94	PI 639240	processing (whole)	Syngenta Seeds Inc.	2007
Renegade	BeanCAP	95	PI 641959	fresh market	Syngenta Seeds Inc.	2007
Rocdor	BeanCAP	96	NA	wax	Vilmorin S.A.	NA
Rockport	BeanCAP	97	PI 653721	processing	Syngenta Seeds Inc.	2008
Roller	BeanCAP	98	NA	fresh market	Seminis Vegetable Seeds Inc.	NA
Roma_II	BeanCAP	99	PI 549997	romano	Rogers Brothers Seed Company	1980
Romano_118	BeanCAP	100	NA	romano	Seminis Vegetable Seeds Inc.	NA
Romano_Gold	BeanCAP	101	PI 634344	romano	Seminis Vegetable Seeds Inc.	2007
Royal_Burgundy	BeanCAP	102	NA	purple	Charter Seed Company	NA
Sapporo	BeanCAP	103	NA	fresh market	Syngenta Seeds Inc.	NA
Scorpio	BeanCAP	104	PI 632268	fresh market	Syngenta Seeds Inc.	2003
Sea_biscuit	BeanCAP	105	PI 642354	fresh market	Seminis Vegetable Seeds Inc.	2008
Secretariat	BeanCAP	106	PI 642316	fresh market	Seminis Vegetable Seeds Inc.	2008
Selecta	BeanCAP	107	NA	NA	Seminis Vegetable Seeds Inc.	NA
Serengeti	BeanCAP	108	PI 660678	fresh market	Syngenta Crop Protection Ag.	2011
Serin	BeanCAP	109	NA	fresh market	Seminis Vegetable Seeds Inc.	NA
Seville	BeanCAP	110	PI 550708	dual	Rogers NK Seed Co.	1992
Shade	BeanCAP	111	NA	fresh market	Harris Moran Seed Company	NA
Sirio	BeanCAP	112	NA	proccessing (whole)	Syngenta Seeds Inc.	NA

Slenderella	BeanCAP	113	PI 550342	fresh market	Ferry-Morse Seed Company	1991
Slenderpack	BeanCAP	114	PI 632692	dual	Seminis Vegetable Seeds Inc.	2005
Sonesta	BeanCAP	115	NA	fresh market	Pop Vriend	NA
Spartacus	BeanCAP	116	PI 642353	processing	Seminis Vegetable Seeds Inc.	2008
Speedy	BeanCAP	117	NA	fresh market	Nunhems Seed Coporation	NA
Stallion	BeanCAP	118	PI 599196	fresh market	Seminis Vegetable Seeds Inc.	2001
Stayton	BeanCAP	119	PI 641958	processing	Syngenta Seeds Inc.	2007
Storm	BeanCAP	120	PI 599323	fresh market	Seminis Vegetable Seeds Inc.	2001
Strike	BeanCAP	121	PI 549970	fresh market	Asgrow Seed Company	1977
Stringless_Frech_Filet	BeanCAP	122	NA	fresh market	NA	NA
Summit	BeanCAP	123	PI 564523	processing	Rogers NK Seed Co.	1993
Tapia	BeanCAP	124	PI 615081	romano	Seminis Vegetable Seeds Inc.	2002
Tendercrop	BeanCAP	125	NA	processing	USDA	NA
Tendergreen	BeanCAP	126	NA	processing	Rogers Brothers Seed Company	NA
Teseo	BeanCAP	127	PI 566908	fresh market	Rogers Seed Co.	1997
Thoroughbred	BeanCAP	128	PI 632448	fresh market	Seminis Vegetable Seeds Inc.	2003
Titan	BeanCAP	129	PI 628336	processing	Seminis Vegetable Seeds Inc.	2002
Top_Crop	BeanCAP	130	NA	proccessing	USDA	NA
Trueblue	BeanCAP	131	PI 550343	processing	Ferry-Morse Seed Company	1991
Ulysses	BeanCAP	132	PI 642359	processing	Seminis Vegetable Seeds Inc.	2008
Unidor	BeanCAP	133	NA	fresh market	Seminis Vegetable Seeds Inc.	withdrawn
Valentino	BeanCAP	135	PI 642321	fresh market	Seminis Vegetable Seeds Inc.	2008
Venture	BeanCAP	136	PI 550279	processing	Rogers Brothers Seed Company	1989
Warrior	BeanCAP	137	PI 628351	fresh market	Seminis Vegetable Seeds Inc.	2002

Widusa	BeanCAP	138	NA	fresh market	IVT	NA
Zeus	BeanCAP	139	PI 606783	processing	Seminis Vegetable Seeds Inc.	2001
Zodiac	BeanCAP	140	NA	fresh market	Seminis Vegetable Seeds Inc.	withdrawn
US_Refugee_5	BeanCAP	141	NA	fresh market	NA	NA
Blue_Peter_Pole	BeanCAP	142	NA	fresh market pole	NA	NA
Corbett_Refugee	BeanCAP	143	PI 549829	fresh market	USDA	NA
Fortex	BeanCAP	144	NA	fresh market pole	INRA	NA
McCaslan_No._42	BeanCAP	145	NA	fresh market pole	Corneli Seed Company	NA
Oregon_Giant_Pole	BeanCAP	146	NA	fresh market pole	Oregon State University	NA
Blue_Lake_Pole	BeanCAP	147	NA	fresh market pole	NA	NA
Blue_Lake_Pole_S7	BeanCAP	148	NA	fresh market pole	Asgrow Seed Company	NA
Trail_of_Tears	BeanCAP	149	NA	fresh market pole	Seed Savers Exchange	NA
Kentucky_Wonder_Pole	BeanCAP	150	NA	fresh market pole	NA	NA
Moncayo	BeanCAP	NA	PI 598219	romano	Novartis Seeds Inc.	2001
Jolanda	CIAT	NA	G 7591	fresh market	NA	NA
Podsquad	CU	NA	PI 550283	fresh market	Asgrow Seed Company	1991
Mirada	CU	NA	PI 561045	fresh market	Rogers Seed Co.	1996
Soleil	CU	NA	PI 590224	wax - dual	Vilmorin S.A.	1999
Beany_Baby	CU	NA	PI 606784	processing (whole)	Seminis Vegetable Seeds Inc.	2001

Indy_Gold	CU	NA	PI 596571	wax - processing	Novartis Seeds Inc.	2001
Capricorn	CU	NA	PI 612348	fresh market	Syngenta Seeds Inc.	2002
Baby_Bop	CU	NA	PI 606781	processing (whole)	Seminis Vegetable Seeds Inc.	2002
Lynx	CU	NA	PI 630927	fresh market	Seminis Vegetable Seeds Inc.	2002
Gold_Ribbon _(Ex_081207 03)	CU	NA	PI 671982	wax-processing	Seminis Vegetable Seeds Inc.	2008
Dynasty	CU	NA	NA	dual	Brotherton Seed Company	2013
Orient	CU	NA	NA	fresh market	Holland-Select Research B.V.	abandoned
Sonata	CU	NA	NA	fresh market	Harris Moran Seed Company	abandoned
Amy	CU	NA	NA	fresh market	Seminis Vegetable Seeds Inc.	NA
Erin	CU	NA	NA	fresh market	NA	NA
Foremost	CU	NA	NA	fresh market	NA	NA
Isar	CU	NA	NA	fresh market	Pop Vriend	NA
Marseille	CU	NA	NA	fresh market	Harris Moran Seed Company	NA
Almaty	CU	NA	NA	NA	Pop Vriend	NA
Barrier	CU	NA	NA	fresh market	Alpha Seed	NA
Bogey	CU	NA	NA	NA	Pop Vriend	NA
Cartagena	CU	NA	NA	proccessing (whole)	Seminis Vegetable Seeds Inc.	NA
Cruiser	CU	NA	NA	dual	Vilmorin S.A.	NA
Freshpick	CU	NA	NA	fresh market	NA	NA
Jubba	CU	NA	NA	fresh market	Pop Vriend	NA
Juliet	CU	NA	NA	fresh market	Pop Vriend	NA
Malibu	CU	NA	NA	fresh market pole	Harris Moran Seed Company	NA
Maxibel	CU	NA	NA	fresh market	Vilmorin S.A.	NA
Molly	CU	NA	NA	fresh market	Nunhems Seed Coporation	NA

Sungold_(CW_198)	CU	NA	PI 549983	fresh market	Cornell University	NA
Volta	CU	NA	NA	fresh market	Pop Vriend	NA
Laureat	CU	NA	PI 550261	processing	Asgrow Seed Company	withdrawn
Tavera	JSS	NA	NA	fresh market	Pop Vriend	NA
Velour	JSS	NA	NA	fresh market	Clause Home Garden	NA
Amythest	JSS	NA	NA	fresh market	Clause Home Garden	NA
Pencil_Pod_Golden_Wax	SSE	NA	NA	wax-dual	NA	NA
Royalty_Purple_Pod	SSE	NA	PI 549644	purple	University of New Hampshire	NA
Burpees_Stringless	SSE	NA	NA	fresh market	W.A. Burpee & Co.	NA
Climbing_French	SSE	NA	NA	fresh market pole	SSE	NA
Romano_Purpiat	Territorial	NA	NA	romano	NA	NA
Blue_Crop	USDA	NA	PI 549926	proccessing	Ferry-Morse Seed Company	1972
Amigo	USDA	NA	PI 549945	KY flat	Ferry-Morse Seed Company	1974
Bush_Blue_Lake_Supreme	USDA	NA	PI 549912	proccessing	Asgrow Seed Company	1974
Lake_Geneva	USDA	NA	PI 549919	processing	Keystone Seed Company	1974
Lake_Largo	USDA	NA	PI 549920	processing	Keystone Seed Company	1974
Lake_Shasta	USDA	NA	PI 549921	processing	Keystone Seed Company	1974
Petite	USDA	NA	PI 549931	processing	Ferry-Morse Seed Company	1974
Rainier	USDA	NA	PI 549932	processing	Ferry-Morse Seed Company	1974
Roma	USDA	NA	PI 549936	romano	Rogers Brothers Seed Company	1974
Valgold	USDA	NA	PI 549909	wax - dual	Gallatin Valley Seed Company	1974
White_Seeded_Provider	USDA	NA	PI 549933	dual	Charter Seed Company	1974

BBL_47	USDA	NA	PI 549911	processing	Asgrow Seed Company	1974
Bush_Romano_71	USDA	NA	PI 549927	romano	Ferry-Morse Seed Company	1974
Checkmate	USDA	NA	PI 549913	proccessing	Asgrow Seed Company	1974
Galamor	USDA	NA	PI 549907	processing	Gallatin Valley Seed Company	1974
Gator_Green_15	USDA	NA	PI 549929	dual	Ferry-Morse Seed Company	1974
Gem	USDA	NA	PI 549935	wax (dual)	FMC Corporation	1974
Lake_Erie	USDA	NA	PI 549918	processing	Keystone Seed Company	1974
Lake_Superior	USDA	NA	PI 549922	processing	Keystone Seed Company	1974
Miami	USDA	NA	PI 549923	processing	Keystone Seed Company	1974
Sinclair_Butterwax	USDA	NA	PI 549905	processing	Agway Inc.	1974
Sunrise	USDA	NA	PI 549934	wax	Keystone Seed Company	1974
Thor	USDA	NA	PI 549917	processing	Asgrow Seed Company	1974
Rodeo	USDA	NA	PI 549943	processing	Asgrow Seed Company	1975
Slenderette	USDA	NA	PI 549947	processing	James L. Musser and C. A. Davenport	1975
Bush_Blue_Lake_53	USDA	NA	PI 549941	processing	Asgrow Seed Company	1975
Greenpak	USDA	NA	PI 549950	proccessing	Rogers Brothers Seed Company	1975
Pax	USDA	NA	PI 549942	processing	Asgrow Seed Company	1975
Spurt	USDA	NA	PI 549944	fresh market	Asgrow Seed Company	1975
Tenderblue	USDA	NA	PI 549946	processing	Ferry-Morse Seed Company	1975
Cape	USDA	NA	PI 549957	processing	Asgrow Seed Company	1976
Century_Gold	USDA	NA	PI 549952	wax	Rogers Brothers Seed Company	1976
Goldette	USDA	NA	PI 549964	wax-processing	James L. Musser and C. A. Davenport	1976

Greensleeves	USDA	NA	PI 549949	proccessing	W. Atlee Burpee Company	1976
Torrent	USDA	NA	PI 549961	processing	Ferry-Morse Seed Company	1976
Aristocrop	USDA	NA	PI 549959	processing	Ferry-Morse Seed Company	1976
Grand_Canyon	USDA	NA	PI 549963	proccessing	Idaho Seed Bean Co. Inc.	1976
Stretch	USDA	NA	PI 549958	fresh market	Asgrow Seed Company	1976
Tidal_Wave	USDA	NA	PI 549960	processing	Ferry-Morse Seed Company	1976
Gabriella	USDA	NA	PI 549968	wax (dual)	Asgrow Seed Company	1977
Gaelic	USDA	NA	PI 549969	proccessing (whole)	Asgrow Seed Company	1977
Triumph	USDA	NA	PI 549980	processing	Agrigenetics Corporation	1977
Early_Blue	USDA	NA	PI 549972	processing	Ferry-Morse Seed Company	1977
Green_Genes	USDA	NA	PI 549973	KY flat	Northrup King and Company	1977
Majestic	USDA	NA	PI 549965	wax - processing	Rogers Brothers Seed Company	1977
Golden_Rod	USDA	NA	PI 549988	wax - dual	Ferry-Morse Seed Company	1978
Lakeland	USDA	NA	PI 549978	processing	Agrigenetics Corporation	1978
Lancer	USDA	NA	PI 549990	proccessing	Rogers Brothers Seed Company	1978
Vitagreen	USDA	NA	PI 549984	processing	Rogers Brothers Seed Company	1978
Early_Bird	USDA	NA	PI 549991	processing	van Waveren-Pflanzenzucht GmbH	1979
Galagold	USDA	NA	PI 550000	wax (processing)	Gallatin Valley Seed Co. Division of Rogers Brothers Seed Co.	1980
BBL_109	USDA	NA	PI 549999	proccessing	Gallatin Valley Seed Company A Division of Rogers Brothers Seed Company	1980
Golden_Sands	USDA	NA	PI 550005	wax - dual	Ferry-Morse Seed Company	1980

Keygold	USDA	NA	PI 550003	wax - processing	Keystone Seed Co. Inc.	1980
Pirate	USDA	NA	PI 550004	KY flat	Asgrow Seed Company	1980
Smilo	USDA	NA	PI 549998	processing (whole)	Royal Sluis B.V.	1980
Win	USDA	NA	PI 550011	processing	Asgrow Seed Company	1981
Frenchy	USDA	NA	PI 550126	proccessing (whole)	Royal Sluis	1981
Peak	USDA	NA	PI 550024	dual	Asgrow Seed Company	1981
Flo	USDA	NA	PI 550023	processing	Asgrow Seed Company	1982
Goldie	USDA	NA	PI 550034	wax	Rogers Brothers Seed Company	1982
Tenderlake	USDA	NA	PI 550053	processing	Ferry-Morse Seed Company	1982
Blue_Duet	USDA	NA	PI 550025	proccessing	Moran Seeds Inc.	1982
Burly	USDA	NA	PI 550033	proccessing	Rogers Brothers Seed Company	1982
Empress	USDA	NA	PI 550031	processing	Agrigenetics Corporation	1982
Epoch	USDA	NA	PI 550022	processing	Wilbur-Ellis Company	1982
Flamata	USDA	NA	PI 550054	flageolet	Royal Sluis B.V.	1982
Jumbo	USDA	NA	PI 550044	romano	Gallatin Valley Seed Co. Division of Rogers Brothers Seed Co.	1982
Score	USDA	NA	PI 550026	processing	Moran Seeds Inc.	1982
Producer	USDA	NA	PI 550051	fresh market	Ferry-Morse Seed Company	1983
Shannon	USDA	NA	PI 550117	processing	Agrigenetics Corporation	1983
Trend	USDA	NA	PI 550128	processing	Royal Sluis	1983
Brokers_Choice	USDA	NA	PI 550115	fresh market	Agrigenetics Corporation	1983
Crossville	USDA	NA	PI 550049	processing	Ferry-Morse Seed Company	1983
Lute	USDA	NA	PI 550127	fresh market	Royal Sluis B.V.	1983

Monaco	USDA	NA	PI 550137	processing	Holland-Select B.V.	1983
Profit_Maker	USDA	NA	PI 550116	fresh market	Agrigenetics Corporation	1983
Flaveol	USDA	NA	PI 550125	flageolet	Royal Sluis	1984
Nomara	USDA	NA	PI 550141	dual	Royal Sluis	1984
Atlantic	USDA	NA	PI 550134	fresh market	Asgrow Seed Company	1985
Bush_Kentucky_Wonder_125	USDA	NA	PI 550130	KY flat	Musser Seed Co. Inc.	1985
Ovation	USDA	NA	PI 550142	proccessing	Royal Sluis	1985
Amity	USDA	NA	PI 550156	fresh market	van Waveren Pflanzenzucht GmbH	1986
Sundial	USDA	NA	PI 550052	wax - dual	Ferry-Morse Seed Company	1986
Tanta	USDA	NA	PI 550257	dual	Wilbur-Ellis Co. Seed Division	1986
Accord	USDA	NA	PI 550155	fresh market	van Waveren Pflanzenzucht GmbH	1986
Caesar	USDA	NA	PI 550048	romano	Ferry-Morse Seed Company	1986
Evergreen	USDA	NA	PI 550157	proccessing	van Waveren-Pflanzenzucht GmbH	1986
Slimgym	USDA	NA	PI 550045	fresh market	Syngenta Seeds Inc. (Rogers)	1986
Shamrock	USDA	NA	PI 550139	fresh market	Rogers Brothers Seed Company	1987
EZ_Harvest	USDA	NA	PI 550254	processing	NPI AgService Corporation	1987
Slenderwax	USDA	NA	PI 550269	dual	Musser Seed Co.	1988
Sentry	USDA	NA	PI 550284	processing	Asgrow Seed Company	1989
BBL_110	USDA	NA	PI 538771	processing	Rogers NK Seed Co.	1990
Goldkist	USDA	NA	PI 550270	wax	Rogers Brothers Seed Company	1990
Stiletto	USDA	NA	PI 550290	processing	Ferry-Morse Seed Company	1990

Blue_Knight	USDA	NA	PI 550289	proccessing	Ferry-Morse Seed Company	1990
Sprout	USDA	NA	PI 550285	fresh market	Asgrow Seed Company	1990
Applause	USDA	NA	PI 550344	proccessing	Asgrow Seed Company	1991
Biscayne	USDA	NA	PI 550345	fresh market	Asgrow Seed Company	1991
Bush_Romano_635	USDA	NA	PI 550411	romano	Rogers NK Seed Co.	1991
Homestyle	USDA	NA	PI 550346	processing	Asgrow Seed Company	1991
Shore	USDA	NA	PI 550154	processing	Ferry-Morse Seed Company	1991
Axel	USDA	NA	PI 550701	processing (whole)	Vilmorin S.A.	1992
Blue_Ridge	USDA	NA	PI 550149	proccessing	Ferry-Morse Seed Company	1992
Bush_Romano_350	USDA	NA	PI 538770	romano	Rogers NK Seed Co.	1992
Crest	USDA	NA	PI 550422	processing	Asgrow Seed Company	1992
DMC_04-04	USDA	NA	PI 560310	processing	Del Monte Corporation	1992
DMC_04-88	USDA	NA	PI 559391	processing	Del Monte Corporation	1992
DMC_04-94	USDA	NA	PI 560312	processing	Del Monte Corporation	1992
DMC_06-01	USDA	NA	PI 560313	romano	Del Monte Corporation	1992
Early_Sunray	USDA	NA	PI 550402	processing	Bakker Brothers of Idaho Inc.	1992
Fesca	USDA	NA	PI 555455	proccessing	Nunhems Seed Coporation	1992
Gentry	USDA	NA	PI 546488	proccessing	Rogers NK Seed Co.	1992
Kentucky_Blue	USDA	NA	PI 539928	fresh market pole	Rogers NK Seed Co.	1992
Minidoka	USDA	NA	PI 539929	fresh market pole	Rogers NK Seed Co.	1992
Mustang	USDA	NA	PI 550425	fresh market	Asgrow Seed Company	1992
Pierre	USDA	NA	PI 548815	processing-whole	Ferry-Morse Seed Company	1992

Primo	USDA	NA	PI 550153	romano	Ferry-Morse Seed Company	1992
Rapids	USDA	NA	PI 544072	processing	Ferry-Morse Seed Company	1992
Castel	USDA	NA	PI 550431	proccessing (whole)	Vilmorin S.A.	1992
DMC_04-34	USDA	NA	PI 560311	processing	Del Monte Corporation	1992
DMC_08-52	USDA	NA	PI 560315	wax	Del Monte Corporation	1992
Satin	USDA	NA	PI 537107	processing (whole)	Ferry-Morse Seed Company	1992
Sunrae	USDA	NA	PI 538769	wax - dual	Rogers NK Seed Co.	1992
Tema	USDA	NA	PI 550426	fresh market	Asgrow Seed Company	1992
Wax_216	USDA	NA	PI 550408	wax - dual	Rogers NK Seed Co.	1992
Wrangler	USDA	NA	PI 538027	processing	Asgrow Seed Company	1992
DMC_04-01	USDA	NA	PI 564075	processing	Del Monte Corporation	1993
Wax_Romano_82264	USDA	NA	PI 561046	romano	Rogers NK Seed Co.	1993
DMC_04-14	USDA	NA	PI 561590	processing	Del Monte Corporation	1993
DMC_04-60	USDA	NA	PI 561931	processing	Del Monte Corporation	1993
DMC_04-61	USDA	NA	PI 561932	processing	Del Monte Corporation	1993
DMC_06-39	USDA	NA	PI 560314	romano	Del Monte Corporation	1993
DMC_08-02	USDA	NA	PI 561592	wax	Del Monte Corporation	1993
Nickel	USDA	NA	PI 578880	dual	Vilmorin S.A.	1997
Leon	USDA	NA	PI 628340	fresh market	Syngenta Seeds Inc.	2003
Daytona	USDA	NA	PI 585237	fresh market	Ferry-Morse Seed Company	abandoned
Early_Riser	USDA	NA	PI 550146	processing	Ferry-Morse Seed Company	abandoned
Salou	USDA	NA	PI 578020	fresh market	Asgrow Seed Company	abandoned
Highway	USDA	NA	PI 578018	NA	Asgrow Seed Company	abandoned

Stride	USDA	NA	PI 550293	fresh market	Gallatin Valley Seed Company	abandoned
Symphony	USDA	NA	PI 590572	NA	Ferry-Morse Seed Company	abandoned
BAT_93	USDA	NA	PI 633451	dry bean	NA	NA
Early_Gallatin	USDA	NA	PI 549847	processing	Gallatin Valley Seed Company	NA
Harvester	USDA	NA	PI 549648	proccessing	Asgrow Seed Company	NA
Ideal_Market	USDA	NA	PI 549527	fresh market pole	NA	NA
IVT_7214	USDA	NA	PI 602987	fresh market	IVT	NA
IVT_7233	USDA	NA	PI 599029	fresh market	IVT	NA
Kentucky_Wonder_Bush	USDA	NA	PI 549544	fresh market	NA	NA
Processor	USDA	NA	PI 549579	processing	Ferry-Morse Seed Company	NA
Refugee_Wax	USDA	NA	PI 554137	fresh market	USDA	NA
Slendergreen	USDA	NA	PI 549561	fresh market	Rogers Brothers Seed Company	NA
Stringless_Green_Refugee	USDA	NA	PI 598999	fresh market	NA	NA
Amanda	USDA	NA	PI 599026	fresh market	IVT (Institute for Horticultural Plant Breeding Wageningen)	NA
Apollo	USDA	NA	PI 549879	dual	USDA	NA
Blue_Mountain	USDA	NA	PI 550122	proccessing	USDA-ARS	NA
Bountiful	USDA	NA	PI 598998	fresh market	University of Idaho	NA
Earligreen	USDA	NA	PI 549617	processing	Rogers Brothers Seed Company	NA
Earliwax	USDA	NA	PI 549618	wax-processing	Rogers Brothers Seed Company	NA
Evolutie	USDA	NA	W6 42706	fresh market	Vilmorin S.A.	NA
Flagrano	USDA	NA	PI 661907	flageolet	NA	NA

Goldcrop	USDA	NA	PI 549903	dual	USDA	NA
Golden_Gate _Wax	USDA	NA	PI 608442	fresh market	USDA	NA
Improved_Te ndergreen	USDA	NA	PI 599024	proccessing	Rogers Brothers Seed Company	NA
Imuna	USDA	NA	PI 326420	fresh market	NA	NA
Jalo_EEP558	USDA	NA	PI 608392	dry bean	NA	NA
Resisto	USDA	NA	PI 549982	fresh market	Rogers Brothers Seed Company	NA
RH13	USDA	NA	W6 28061	Breeding line	INRA	NA
Slimgreen	USDA	NA	PI 549630	fresh market	Rogers Brothers Seed Company	NA
Splendergold	USDA	NA	PI 549955	fresh market	Rogers Brothers Seed Company	NA
Sprite	USDA	NA	PI 550248	fresh market	NA	NA
Wondergreen	USDA	NA	PI 549956	processing	Rogers Brothers Seed Company	NA
Blazer	USDA	NA	PI 550258	processing	Rogers Brothers Seed Company	withdrawn
Bluepak	USDA	NA	PI 550259	processing	Rogers Brothers Seed Company	withdrawn
Bounty	USDA	NA	PI 550145	proccessing	Ferry-Morse Seed Company	withdrawn
Duchess	USDA	NA	PI 542389	fresh market	Novartis Seeds inc.	withdrawn
Flevoro	USDA	NA	PI 561588	fresh market	Seminis Vegetable Seeds Inc.	withdrawn
Legion	USDA	NA	PI 550423	processing	Asgrow Seed Company	withdrawn
Mikado	USDA	NA	PI 550136	fresh market	UF Genetics Inc.	withdrawn
Mount_Hood	USDA	NA	PI 550251	proccessing	Ferry-Morse Seed Company	withdrawn
Allure	USDA	NA	PI 561587	NA	Seminis Vegetable Seeds Inc.	withdrawn
Bonanza	USDA	NA	PI 549877	processing	Gallatin Valley Seed Company	withdrawn
Clyde	USDA	NA	PI 583286	proccessing (whole)	Rogers Brothers Seed Company	withdrawn
Modus	USDA	NA	PI 554607	fresh market	Nunza B.V.	withdrawn

Quest	USDA	NA	PI 583361	fresh market	Harris Moran Seed Company	withdrawn
Saratoga	USDA	NA	PI 599576	NA	Seminis Vegetable Seeds Inc.	withdrawn
Savor	USDA	NA	PI 550252	fresh market	Moran Seeds Inc.	withdrawn
Surfing	USDA	NA	PI 550143	fresh market	Mitsui Toatsu Chemicals Inc.	withdrawn
Tempest	USDA	NA	PI 572549	NA	Harris Moran Seed Company	withdrawn
Yukon	USDA	NA	PI 550287	dual	Asgrow Seed Company	withdrawn