ASCORBIC ACID CONTENT IN SPINACH:

PHENOTYPIC DIVERSITY, ASSOCIATION MAPPING, AND EFFECT ON

SALT STRESS TOLERANCE

A Thesis

by

DARIO DIOGENES RUEDA KUNZ

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Chair of Committee,	Carlos A. Avila
Co-Chair of Committee,	Kevin Crosby
Committee Members,	Patricia Klein
	Kranthi Mandadi
Head of Department,	Patricia Klein

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ABSTRACT

Spinach (*Spinacia oleracea*) is an economically important leafy vegetable crop. Ascorbic acid (AsA), or vitamin C, is an essential nutrient for humans, involved in growth, development, and tissue repair. In plants, AsA functions as an antioxidant eliminating cell-damaging free radicals produced during normal metabolism or in response to stress. The increase of AsA content in model plants has resulted in abiotic stress tolerance. However, AsA content diversity in spinach germplasm is not well-known. In this study, we performed AsA quantification in a worldwide panel of 352 spinach accessions and determined that variability in AsA content in spinach germplasm is high and could be utilized for cultivar improvement. In addition, a genome-wide association study was performed using the Generalized linear model (GLM), compressed mixed linear model using P3D, and the perMarker model. A total of 490 SNPs resulted in significant marker-to-trait associations between all models. SNP markers were distributed in all six spinach chromosomes. Among the identified SNPs, only 27 were detected by all three models, commonly identified markers can be used as primary targets for validation. Identified candidate genes linked to the SNPs, did not include any known AsA biosynthesis pathway genes, suggesting that the observed AsA level differences may be related to substrate availability, the effect of plant hormones, or differences in transcriptional regulation. We also evaluated the effect of AsA on salt stress tolerance in ten spinach lines with low and high AsA contents. Plants were treated with a 150 mM NaCl solution. Stomatal conductance was higher in plants with high AsA content, showing a positive relationship when plants were under salt stress and higher

chlorophyll fluorescence values independent of salt stress. These results suggest that AsA allows spinach plants to better tolerate salt stress by maintaining photosynthesis and respiration, although it was not reflected in a significant effect on biomass production at 150 mM NaCl. Finally, *in silico* analysis indicated that identified markers can differentiate between high and low AsA content accessions and that, upon validation, these markers should be useful for breeding programs.

DEDICATION

To my family: misai, pisao, felipiro y dieguitoperi. Thanks for always been there even when I made mistakes you always had my back. You kept motivating me whenever I need it and you kept pushing me when I had doubts of myself. Of course, all this on your own Rueda Kunz style. Thanks dad and mom, for never setting any desired expectations in my life and let me be who I thought I needed to be; I am sure you both knew I was going to triumph on my own pace.

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NOMENCLATURE

NaCl	Sodium Chloride
cv	cultivar
Mb	mega base pairs
kb	kilo base pairs
Lbs	pounds
M	Millions
DV	daily value
IU	International unit
mg	milligram
mcg	microgram
AsA	Ascorbic acid
QTL	quantitative trait loci
dS	deciSiemens
mM	millimolar
Na	Sodium
K	Potassium
Ca	Calcium
ROS	Reactive oxygen species
SNP	single nucleotide polymorphism
UV	ultraviolet
NILs	near inbred lines

SD standard deviation

mL milliliter

C° Celsius

Chr Chromosome

GWAS Genome-wide association study

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CHAPTER I

INTRODUCTION

1.1 Origin of spinach and botanical classification

Spinach (*Spinacia oleracea* L.) is a leafy green that belongs to the Chenopodiaceae family. It is an annual diploid plant (2n = 2x = 12) with an estimated genome size of 989 Mb (Arumuganathan and Earle, 1991). Spinach is a mostly dioecious plant, where female and male reproductive organs are not found in the same plant (Awika et al., 2020), although monoecious plants are also present in low frequency among spinach species (Khattak et al., 2006).

The center of origin for spinach is thought to be in Asia, specifically in Persia in what is now known as Iran. Although the dissemination of spinach towards China and Europe is clear, the routes for this are still unclear (Morelock and Correll, 2008, Ribera et al., 2020). Cultivated spinach has two wild relatives, *S. turkestanica* and *S. tetrandra*. These wild relatives are of interest for breeders as a source of new traits for cultivar improvement (Ribera et al., 2020). In the United States, spinach was first introduced by the European colonists, who had been growing it since the 11th century (Ribera et al., 2020, Correll et al., 2011, Kandel et al., 2019, Klockow and Keener, 2009). As a leafy green it is consumed fresh (Klockow and Keener, 2009), cooked, or as part of other dishes (Kandel et al., 2019, Morelock and Correll, 2008). For humans, spinach consumption can be beneficial due to its high nutritional content. For example, spinach is high in vitamins A and C, as well as in several minerals including calcium, iron and

sodium (Younis et al., 2015). Spinach also contains high levels of flavonoids, and the carotenoids, lutein and zeaxanthin (Ribera et al., 2020).

1.2 Spinach economic importance

Spinach is an economically important leafy green crop. In 2019, a total of 65,800 acres were harvested in the US and most of the production was concentrated in California, Arizona, Texas, and New Jersey (USDA-NASS, 2020). Acreage has been increasing ~8% per year over the last seven years, with 61,450 acres valued at $422.9 M in 2018. California leads the US market with 75% of sales, of which 35-55% is organically grown. In Texas, in the year 2018 an area of 2,800 acres were harvested, with a production value of $6.1 M (USDA-NASS, 2019).

In the US, the per-capita consumption of fresh spinach increased from 1.73 lbs- to 2.48 lbs-*per capita* between 2015 and 2019, respectively, indicating the rise in popularity of fresh spinach produce. On the other hand, canned spinach had a decrease from 0.15 lbs- to 0.13 lbs-*per capita* during the same time period (USDA-ERS, 2020).

1.3 Spinach nutritional content

Spinach is a highly nutritious leafy green. It has most of the essential vitamins our body requires, such as vitamins A, C, E and K; and is also abundant in minerals (Table 1.1). One serving of spinach provides from 3 to 13% of the recommended daily value for calcium, iron, magnesium, potassium, and manganese (Table 1.1). Spinach nutritional content exceeds that of lettuce, the most popular leafy green, in both vitamins

and minerals (Table 1.1). Furthermore, spinach has high levels of health promoting compounds (e.g., lutein, zeaxanthin, ascorbic acid) that benefit eye health, and reduce oxidative stress, among other roles (Chung et al., 2019). Optimizing these nutrient concentrations together with consumer-preferred texture, color, and taste will further promote spinach and leafy green consumption.

Table 1.1 Nutrient profiles for 1 cup of spinach and crisphead/iceberg lettuce (https://nutritiondata.self.com/)

		Spinach		**Crisphead lettuce**	
		Amount/serving	%DV	Amount/serving	%DV
Vitamins	Vitamin A	2813 IU	56%	361 IU	7%
	Vitamin C	8.4 mg	14%	2.0 mg	3%
	Vitamin D	0.0 mg	0 %	0.0 mg	0 %
	Vitamin E	0.6 mg	3%	0.1 mg	1%
	Vitamin K	145 mcg	181%	17.4 mcg	22%
	Thiamin	0.0 mg	2%	0.0 mg	2%
	Riboflavin	0.1 mg	3%	0.0 mg	1%
	Niacin	0.2 mg	1%	0.1 mg	0%
	Vitamin B6	0.1 mg	3%	0.0 mg	2%
	Folate	58.2 mcg	15%	20.9 mcg	5%
	Vitamin B12	0.0 mcg	0 %	0.0 mcg	0%
	Choline	5.4 mg			
	Betaine	165 mg			
Minerals	Calcium	29.7 mg	3 %	13.0 mg	1%
	Iron	0.8 mg	5 %	0.3 mg	2%
	Magnesium	23.7 mg	6 %	5.0 mg	1%
	Phosphorus	14.7 mg	1 %	14.4 mg	1%
	Potassium	167 mg	5 %	102 mg	3%
	Sodium	23.7 mg	1 %	7.2 mg	0%
	Zinc	0.2 mg	1 %	0.1 mg	1%

	Copper	0.0 mg	2 %	0.0 mg	1%
	Manganese	0.3 mg	13 %	0.1 mg	4%
	Selenium	0.3 mcg	0 %	0.1 mcg	0%

1.4 Biotic and Abiotic stress in spinach production

Spinach production is constantly challenged by abiotic and biotic stresses that result in yield and quality reduction (Agarwal et al., 2018, Feng et al., 2018, Lyon et al., 2016, Min et al., 2018). Therefore, one current goal in spinach breeding programs is to increase crop productivity by improving disease resistance and environmental stress tolerance while enhancing quality and nutritional content.

1.4.1 Spinach diseases

Current breeding efforts are focused on developing resistant cultivars to the most yield-limiting diseases in spinach production including Downy mildew (DM), White Rust (WR), Stemphylium leaf spot (SLS), and Anthracnose (ALS).

Downy mildew, caused by *Peronospora farinose* f. sp. *spinaciae,* is the main disease affecting spinach production (Feng et al., 2014). DM was first reported in spinach in 1885 (Smith, 1885), with the appearance of three races until 1991 (Brandenberger et al., 1991). Since then, new races have appeared more frequently, with the report of at least 12 additional races from 1996 to 2017 (Feng et al., 2018). The appearance of new races is believed to be related to current high-density planting for baby leaf spinach production and continuous production year-round. Furthermore, the

use of race-specific resistant cultivars has systematically increased the selection pressure of the pathogen and resulted in the appearance of resistant races (Correll et al., 2011, Feng et al., 2014). The resistance in spinach against downy mildew is conferred by race-specific single-genes (Correll et al., 1994). So far, nine resistance loci against the 16 races of the pathogen have been identified (Feng et al., 2018). Unfortunately, none of the nine loci provide resistance to all races (Feng et al., 2014).

The second disease in order of importance in the US is the oomycete White Rust (WR) (*Albugo occidentalis*) (Morelock and Correll, 2008). Optimal environmental conditions for disease development include cold nights with high humidity and sunny, dry and hot days (Correll et al., 1994). WR was first reported affecting spinach in 1910 (Brandenberger et al., 1994), although it didn't become a problem in Texas and Arkansas until 1937 and 1945, respectively (Correll et al., 1994). Initially, white rust in spinach, was only reported in the US (Correll et al., 2011). Recent articles show evidence of white rust affecting spinach in Greece (Vakalounakis and Doulis, 2013), Turkey (Soylu et al., 2018) and Mexico (Correll et al., 2017) as well. In contrast to DM, identified white rust resistance in spinach is controlled by several genes (Morelock and Correll, 2008). There are two main types of resistance used in breeding, horizontal and vertical resistance. Horizontal resistance is controlled by a group of genes and will act against more races of the disease while vertical resistance is only controlled by a single gene with the possibility of becoming ineffective with multiple pathogen races or mutations (Van Der Plank, 1966). While horizontal resistance is more difficult to introgress into commercial cultivars, it has a presumptive higher persistence in the field

and is less likely to be affected by race-specific mutations in the pathogen (Brandenberger et al., 1994). Although specific genes involved in white rust have not been reported, there are several spinach cultivars which have been reported as partially resistant to the disease (Goreta and Leskovar, 2006), while some of these cultivars also show some resistance to specific DM races (Correll et al., 2011).

1.4.2 Spinach pests

In addition to plant diseases affecting spinach production, spinach production is challenged by several pests that can cause significant yield and quality losses. One of the most important pests in spinach production is the green peach aphid (*Myzus persicae* Sulzer) which can reduce yield but mostly affects post-harvest processing due to the need to clean the spinach leaves of aphids (Mcleod et al., 1998, Sweeden and McLeod, 1997).

Another insect pest in spinach is the leafminer (*Liriomyza langei*). The wounds caused by the adult and larvae produce a decrease in photosynthesis that results in a significant reduction in yield. Moreover, leaf damage results in unmarketable product due to reduction in aesthetic quality (Mou and Ryder, 2003). Fortunately, resistant cultivars to leafminer are available (Mou, 2008). Recent efforts have identified five possible molecular markers related with resistance against leafminer in spinach, opening up the possibility of marker-assisted selection (Shi and Mou, 2016).

1.4.3 Breeding for abiotic stress tolerance

Climate change is one of the most discussed topics these days. With it we face environmental changes that are affecting agriculture worldwide. Therefore, breeders need to enhance plant resilience towards those changes. Unfortunately, not much attention has been put into improving spinach resilience to abiotic stress. Therefore, targeted breeding efforts against abiotic stresses are needed to enhance spinach tolerance to salt, drought and high and low temperatures.

Temperature stress: spinach is a temperate crop that grows better when the temperature is consistently below 23°C, although cold stress may result when plants are grown in temperatures below 10°C. It has been shown that low temperature (10°C) treatments for seven days can have a beneficial effect on nutritional composition in leaves, increasing vitamin C content three times higher as compared to non-exposed plants (Proietti et al., 2009).

In contrast, above optimal temperatures have a more detrimental effect on plant growth. High temperatures in combination with long photoperiods results in spinach bolting which reduces commercial value (Chitwood et al., 2016). Several breeding efforts have been performed to identify germplasm tolerant to bolting as well to identify molecular markers linked to this trait for use in molecular breeding (Awika et al., 2019, Chitwood et al., 2016).

In addition to breeding efforts, molecular characterization of heat stress in spinach has been reported. The spinach variety ‘Island’ was used to study the response to high temperature (Yan et al., 2016). The authors identified several differentially

expressed genes (DEGs), including heat shock proteins along with other genes related with other abiotic stresses such as oxidative stress after a Gene Ontology and pathway enrichment analysis was performed. These genes can be potentially used for spinach improvement through genetic engineering approaches or by conventional breeding selection.

Drought resistance: Drought responses in spinach have also been studied, although breeding efforts are limited, with most of the efforts focused on the characterization of agronomic practices to reduce the detrimental effects of drought. For example, Zuccarini and Save (2016) reported that the use of mycorrhizaes can significantly improve the drought stress response. These authors showed a gradual decrease of stomatal conductance after 5 days of absence of water when mycorrhizaes were present as compared to the control. In addition to stomatal conductance, mycorrhizaes resulted in fresh weight and leaf area increases in inoculated plants (Zuccarini and Save, 2016).

Likewise, the impact of antioxidative systems, osmoprotectants, chlorophyll pigments and their overall role on plant growth in spinach has been studied (Jabeen et al., 2019). The authors report that after exposing spinach plants to several water levels (40%, 60%, 80% and 100% field capacity) the biomass and chlorophyll content of the plants was negatively impacted, although the content of AsA, proline, glycinebetaine, total phenolics, malondialdehyde and the activities of antioxidant enzymes increased in some of the water stress treatments.

Finally, functional genomic studies have shown that when the spinach gene SoCYP85A1, encoding a cytochrome p450 protein, was incorporated into tobacco plants it resulted in a positive response against drought stress. This was accomplished by eliminating reactive oxygen species, the upregulation of other genes involved in drought stress and the increase of root growth (Duan et al., 2017). These results suggest that drought stress tolerant spinach genes can be used for cultivar improvement.

Nitrogen-use efficiency: Since spinach is a high nitrogen demanding crop, breeding in favor of nitrogen use efficiency (NUE) is a major interest for spinach breeders. For this reason, single nucleotide polymorphism markers associated with quantitative trait loci related with NUE were identified for their use in breeding programs (Chan-Navarrete et al., 2016). Nitrogen use efficiency has also been studied in lettuce, resulting in several QTLs associated with NUE although no gene has been identified that is linked to this trait (Kerbiriou et al., 2016). In another study, lettuce plants overexpressing *LsNRT2.1* gene had better nitrogen use efficiency. (Paez-Valencia et al., 2013). In addition, it has been shown that the use of alternative sources of nitrogen can help to improve NUE. For example, application of calcium cyanamide enhanced the NUE in lettuce (Di Gioia et al., 2017).

1.4.3.1 Salinity stress in spinach

Salinity and water shortage are big concerns in vegetable production regions around the world. Spinach producers have been forced to reduce fresh water irrigation and replace it with saline water with a concomitant increase in salt stress (Ors and

Suarez, 2017). Therefore, breeding, crop management, and physiological response strategies have been focusing on enhancing spinach tolerance to salt stress.

Ors and Suarez (2017) performed a study to evaluate spinach stress responses when exposed to water stress, salt stress and the combination of both. They found that when plants are not drought-stressed but had applied thru the irrigation system a slight (4.0 dS m−1) salinity level the biomass is significantly higher as compared to well-watered control plants. But when more saline water is added, spinach biomass as well as dry weight, shoot height, root weight, leaf number and leaf area are significantly reduced.

The salt stress response of spinach plants was also studied *in vitro* (Muchate et al., 2019). Spinach shoot cultures were exposed to 100mM, 200mM, and 300mM NaCl and several variables were measured, including growth parameters, antioxidant activity of enzymes involved in salt stress, osmotic regulation, oxidative damage caused by reactive oxygen species (ROS) and the content of Na^{+}, K^{+} and Ca^{+2} ions. All the growth parameters were significantly impacted by all salt stress treatments in comparison with the controls. However, while a reduction in growth was observed, the spinach shoot cultures were able to survive *in vitro* indicating some level of tolerance. A greater value was obtained with the highest salt concentrations in terms of most of the antioxidant enzymes with the exclusion of Ascorbate peroxidase and Glutathione reductase, whose highest activity was at 200mM NaCl. Glycine betaine, an osmotic regulator, had its highest value when 300mM NaCl was present in the media. The other two osmotic

regulators, proline and total soluble sugar, were significantly highest at 200mM NaCl. The authors (Muchate et al., 2019) speculated that these increases are due to maintaining the appropriate internal osmotic balance of the plant cells. Relative electrolytic leakage and malondialdehyde content were also measured in order to obtain data about the impact of ROS in this study and they showed a significant increase at higher applied salt concentrations. The authors mentioned that this result was not unexpected since the formation of ROS has a correlation with salinity stress. The ion content in the *in vitro* shoot cultures showed that when applying more salt into the media the concentration of K^+ and Ca^{+2} was significantly lower than the controls and the opposite occurred with Na^+ ions (Muchate et al., 2019).

Other crops like rice (*Oryza sativa*) have also been studied as a model for salt stress to understand root responses. Wang and co-authors identified a prominent gene (*OsVTC1-3*) involved in the synthesis of ascorbic acid in roots conferring tolerance to salt stress. Functional analysis found that knocking-down *OsVTC1-3* resulted in enhanced susceptibility to salt stress, since the ascorbic acid-deficient plants were not able to counteract the detrimental effect of ROS. When applying exogenous AsA to those knocked-down *OsVTC1-3* lines, the effect was reversed resulting in a significant increase of the plant survival rate under salt stress (Wang et al., 2018).

1.4.4 Breeding for quality and nutritional content

In addition to the need of breeding for disease resistance, there is a rising interest among consumers in food attributes in terms of appearance, flavor, and nutritional

density. The “nutrient-dense” term indicates that nutrients and other beneficial substances in a food have not been “diluted” by the addition of calories from added or natural solid fats, sugars, or refined starches in the food (HSS, 2015, USDA, 2015).

However, improving nutritional content has not been prioritized in commercial spinach varieties as breeding for yield and disease resistance have been a predominant focus. Fortunately, natural variation for nutritional content and quality attributes are readily available in spinach germplasm. Recent efforts have been focused to identify parental sources for improvement along with the development of molecular markers associated with quality traits. Qin et al. (Qin et al., 2017), screened spinach germplasm identifying SNP markers associated with the content of 14 minerals. Similarly, accessions with low oxalic acid content and associated molecular markers in spinach were identified. It is believed that high consumption of this chemical can relate to kidney stones in humans (Shi et al., 2016b). In addition, leaf morphology quality traits including surface texture, petiole color and edge shape were also associated with molecular markers (Ma et al., 2016).

1.4.5 Breeding for multiple stress resistance and tolerance

To date, breeding spinach cultivars for nutritional improvement combined with yield, disease resistance, and stress tolerance has been very limited. This is in part due to the complexity of inheritance of some of those traits. Oftentimes, the traits are controlled by quantitative trait loci (QTL). QTL refers to loci that encompass multiple genes governing a particular agronomic trait. QTL are sometimes influenced by plant

developmental stage and environmental factors to ultimately result in stress tolerance. Mapping of such QTL is an invaluable tool for spinach breeding. Furthermore, these traits need to be pyramided together. This is often challenging, because pyramiding traits is labor intensive and time-consuming, and requires screening large populations.

As an alternative to pyramiding several traits, plant central stress regulators, defined as core genes that respond and integrate multiple stress signals to impart tolerance, offer the possibility to reduce the complexity of breeding for several traits at the same time (Avila et al., 2017). Preliminary work on the model plant, *Arabidopsis thaliana* and several crops including potato and rice have identified that in addition to the nutritional benefits for human consumption, high Ascorbic Acid (AsA, also known as vitamin C) increases biomass (higher yield), and enhances tolerance to salinity, cold, and heat stress (Lorence et al., 2004). Therefore, it might be possible to enhance nutritional content and at the same time improve tolerance to abiotic stresses by enhancing ascorbic acid content in spinach.

1.5 Role of Ascorbic acid in plants

Ascorbic acid has two main roles in plants. First, it works as a potent antioxidant eliminating free radicals such as hydrogen peroxide, singlet oxygen or ozone in response to stress that otherwise can cause oxidative damage to the plant cells. The consequences of oxidative cell damage are mostly related to decreased photosynthetic efficiency (Veljović-Jovanović et al., 2017). If the stresses are severe, the antioxidative ability to fight back the oxidation injury is surpassed. In spinach, high ascorbic acid content has

been reported to be involved as part of the plant defense mechanism to reduce the negative impact of ROS under low temperature conditions. The reduction of the temperature can produce changes in the metabolism of spinach leaves, leading to an increase of photosynthesis and a more specific offensive against reactive oxygen species (Proietti et al., 2009).

Second, AsA takes part in several plant physiological and metabolic processes (Gao et al., 2011). Ascorbic acid is responsible for conferring electrons towards Ascorbate peroxidase activity. In conjunction, Ascorbate peroxidase and AsA are essential pieces in the AsA-glutathione cycle (Shigeoka and Maruta, 2014), as well as metabolic functions in cell development (Smirnoff and Wheeler, 2000). Furthermore, AsA plays a role in other processes including redox signaling, reactions against pathogens, flowering induction, hormone formation and defense activation (Yoshimura and Ishikawa, 2017).

1.5.1 Ascorbic acid synthesis in plants

As shown in Figure 1.1 there are four pathways that contribute to the synthesis of ascorbate in plants (Lorence et al., 2004, Suza et al., 2010). These include: 1) the Galacturonase (GalU) pathway where ascorbate is produced from D-Galacturonate when it is synthesized into L-Galactonate and L-Galactono-1,4-lactone. This pathway has been linked to an increase in ascorbic acid content in ripening fruits (Akram et al., 2017, Smirnoff, 2011); 2) The *myo*-inositol (MI) route which is an important supplier of D-Glucuronate that is converted into L-Gulonate and then L-Gulono-1,4-lactone (Lorence et al., 2004); 3) The gulose (Gul) pathway consisting of GDP-L-Gulose via L-Gulose

and L-Gulono-1,4-lactone; and 4) the mannose/galactose (Man/Gal) pathway starting through D-Glucose-6-P then via GDP-D-Mannose, L-Galactose-1-P and L-Galactono-1,4-lactone (Conklin et al., 1999, Smirnoff, 2011, Wheeler et al., 1998).

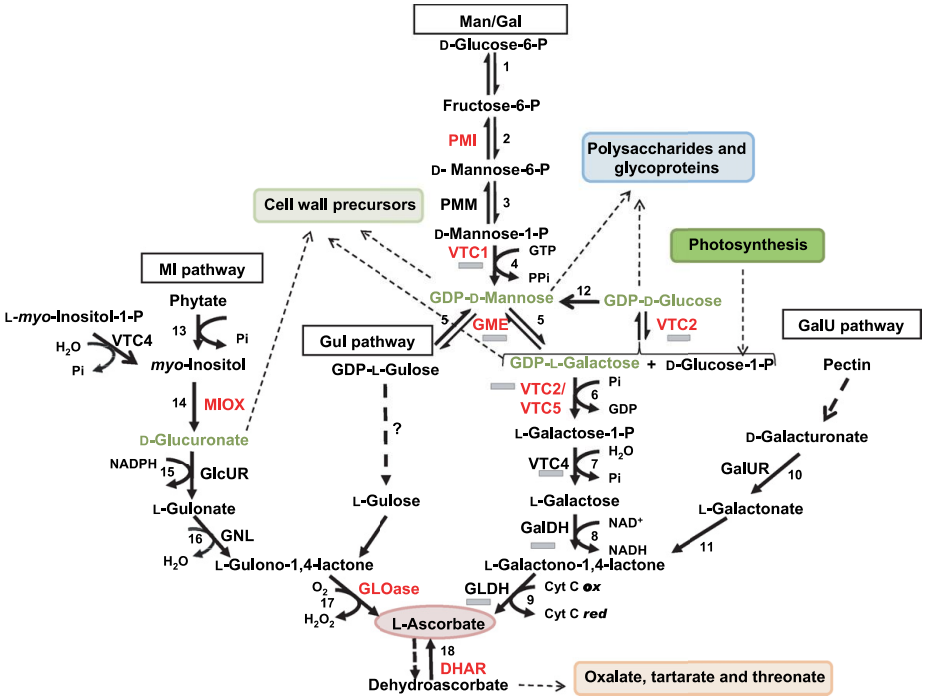

Figure 1.1 Pathways involved in ascorbic acid biosynthesis. The pathways shown are: the mannose/ galactose (Man/Gal) route, the gulose (Gul) shunt, the galacturonase (GalU) pathway, and the myo-inositol (MI) route (Suza et al., 2010).

1.6 Enhancement of Ascorbic acid in plants

1.6.1 Genetic engineering approaches

As an alternative to conventional breeding methods, genetic engineering efforts targeting different enzymes in the ascorbic acid pathway have resulted in enhanced ascorbic acid content in different crops. For example, overexpression in tobacco and maize of a dehydroascorbate reductase (DHAR) showed that ascorbic acid content can

be increased by recycling ascorbic acid from its oxidized state (dehydroascorbate) to the ROS scavenging active state (L-ascorbate) (Chen et al., 2003). Likewise, overexpressing different enzymes in the AsA synthesis pathway such as *myo*-inositol oxidase (MIOX) in Arabidopsis (Lorence et al., 2004) and *GalUR* (D-Galacturonic acid) in potato (Upadhyaya et al., 2011) also resulted in enhanced AsA content. The ascorbic acid enriched transgenic potato plants were shown to have better tolerance against soils with high salinity (Upadhyaya et al., 2011). Qin et al.(Qin et al., 2011) generated a transgenic potato overexpressing two dehydroascorbate reductase genes, which led to a significant increase in Ascorbic acid in tubers and leaves. In addition, the GDP-D-mannose pyrophosphorylase gene from tomato was inserted in tobacco plants resulting in 2 to 4 times higher ascorbate content as compared to wild type plants. Plants with higher AsA content showed reduced accumulation of reactive oxygen species and higher photosynthesis rates when plants were exposed to both low and high temperatures (Wang et al., 2011).

In Arabidopsis, ascorbic acid enhancement was accomplished when the enzyme L-Galactose-1-P Phosphatase, which produces L-Galactose, was upregulated. The enhancement was also obtained through a knockout of ascorbic oxidase in Arabidopsis transgenic plants since it is responsible of the catabolism of L-ascorbate acid into Monodehydro-L-Ascorbate acid (Xiao et al., 2015). In a separate study, the gene *GmGMP1* was found to be triggered in soybean under stresses like temperature, drought, salinity and UV light. For this reason, it was overexpressed in Arabidopsis and soybean (*Glycine max*) with the end result being an increased amount of ascorbic acid in leaves.

Also, it was demonstrated that the transgenic seedlings had better tolerance against osmotic and salt stresses (Xue et al., 2018). Lisko et al.(Lisko et al., 2013b) have worked with myo-inositol oxygenase and L-gulono-1,4-lactone oxidase as an alternative for enhancing AsA content in Arabidopsis. When those genes are overexpressed resulting in higher ascorbic acid, an increase in development along with more biomass in the leaves and roots occurs. Likewise, as we reported in other studies higher ascorbic acid content also enables the capacity of the plant to withstand against abiotic stresses (Lisko et al., 2013b).

Other examples of enhanced ascorbate in plants come from studies with tomato, strawberry, and potato. In tomato and strawberry, a gene from *Actinidia chinensis* was overexpressed, which is named *Actinidia chinensis* GGP (GDP-L-galactose phosphorylase) and is involved in ascorbic acid pathways (Bulley et al., 2012). On the other hand, with potato, an Arabidopsis GGP was overexpressed (Bulley et al., 2012). For these experiments almost all the lines used showed an increase in ascorbic acid. However, the overexpressing tomato lines lacked seed inside the fruit and the locules were empty, indicating that AsA enhancement can result in non-desirable traits due to pleotrophic effects (Bulley et al., 2012). Alternatively, transgenic tomatoes were produced by the overexpression of three genes related with the production of ascorbic acid. The yeast-derived GDP-mannose pyrophosphorylase (GMPase), arabinono-1,4-lactone oxidase (ALO) and myo-inositol oxygenase 2 (MIOX2) from *Arabidopsis thaliana* were used in the transformation of tomato plants, resulting in an increase of

AsA in leaves and fruits indicating the relative importance of the AsA synthesis pathways (Cronje et al., 2012).

A sweet potato gene encoding L-Galactono-1,4-lactone (GalL) dehydrogenase (GLDH), was over-expressed in tobacco plants (Imai et al., 2009). The transgenic lines had increased activity in GLDH in the roots, but not in the shoot or leaves, thus the ascorbic acid content was not higher. When the plants were given a L-Galactono-1,4-lactone solution the ascorbic acid content increased in both transgenic and non-transgenic plants. These results gave rise to the hypothesis that even if a gene is over expressed it will not necessarily result in an increase in biosynthesis of ascorbic acid if substrate is the limiting factor (Imai et al., 2009).

Finally, using a mammalian L-gulono-c-lactone oxidase gene from rats (*Mus musculus*), potato transgenic lines showed an enhanced level of ascorbic acid content. Tubers from these potatoes had 2-times more ascorbic acid content as well as the enzymatic activity when compared to non-transgenic controls. Similar to other studies, the transgenic potatoes were also tolerant to several abiotic stresses (Hemavathi et al., 2010).

1.6.2 Conventional breeding approaches

Conventional breeding has also been used to enhance ascorbic acid in plants. Tomato breeding NILs were developed carrying the genes *hp1* (high pigment 1) and *hp2* (high pigment 2), in which lines having either of those genes showed enhanced ascorbic

acid content along with the creation of a purple tomato fruit and not affecting yield (Sestari et al., 2014). After finding ILs (Introgression lines) harboring QTL's for AsA and phenols in *Solanum pennellii*, F_3 tomato breeding lines were developed and showed a significant increase in ascorbate content compared to the control (Sacco et al., 2013). Five hybrids of chili pepper, created using cytoplasmic male sterility and restorer lines which where resistant or susceptible to several diseases, showed significantly higher amounts of AsA than the commercial controls (Meena et al., 2020). Peach genotypes, produced from crossing two commercial cultivars, were selected after showing enhanced ascorbic acid content and resistance towards brown rot (Obi et al., 2019).

CHAPTER II

ASCORBIC ACID QUANTIFICATION AND GENOME WIDE ASSOCIATION STUDY

2.1 Introduction

Vegetable consumption has significantly increased over the last 20 years (FAOSTAT, 2021), due in part to rising consumer interest in their nutritional properties (Blekkenhorst et al., 2018, Wagner et al., 2016). Spinach (*Spinacia oleracea* L.), of the Chenopodiaceae family, is an economically important, green, leafy crop grown around the world (Schreinemachers et al., 2018). Generally, improving nutritional content has not been prioritized in commercial spinach varieties; the predominant focus has been breeding for yield and disease resistance (She et al., 2018, Kunicki et al., 2010, Villarroel-Zeballos et al., 2012).

Spinach is an annual, diploid plant (2n =2x= 12) with an estimated genome size of 989 Mb (Arumuganathan and Earle, 1991). It is thought to have originated in Asia, specifically in Persia, in what is now Iran. Although it is clear that spinach disseminated towards China and Europe, the specific routes for this migration are still unclear (Morelock and Correll, 2008, Ribera et al., 2020). In the United States, spinach was first introduced in the 11th century by European colonists (Ribera et al., 2020, Correll et al., 2011, Kandel et al., 2019, Klockow and Keener, 2009). As a leafy green, it is consumed fresh (Klockow and Keener, 2009), cooked, or as part of other dishes (Kandel et al., 2019, Morelock and Correll, 2008). Spinach consumption can be beneficial to humans

due to its high nutritional content. For example, spinach is high in vitamins A and C, as well as in several minerals including calcium, iron, and sodium (Younis et al., 2015). Spinach also contains high levels of flavonoids and the carotenoids lutein and zeaxanthin (Ribera et al., 2020).

Vitamin C (ascorbic acid, AsA) is an essential nutrient for humans that is involved in growth, development, and repair of tissues (Padayatty and Levine, 2016). Ascorbic acid is important for humans, as it can prevent scurvy and work as an enzyme cofactor for metabolite catabolism (Fenech et al., 2019). In plants, ascorbic acid functions as an antioxidant that eliminates cell-damaging free radicals produced during normal metabolism or in response to stress (Paciolla et al., 2019). In addition, ascorbic acid is a cofactor in plant signaling pathways related to flowering, detecting and responding to pathogen activity, and modulating the expression of metabolic genes (Ishikawa et al., 2018).

Due to its importance, researchers have aimed to improve AsA content in several crops (Bao et al., 2016, Hemavathi et al., 2009, Zhu et al., 2014, F. Abdelgawad et al., 2019, Kim and Lee, 2016, Kang et al., 2020). To facilitate molecular breeding approaches, several groups have used genome-wide association studies (GWAS) to explore germplasm variation and to identify associations between genetic variation and phenotypic data (Tam et al., 2019). For example, a study performed in apple (*Malus* x *domestica*) revealed significant variations in AsA content found in fruit, although no significant genetic markers were associated with AsA (Lemmens et al., 2020). By contrast, a GWAS revealed 22 significant markers associated with AsA in tomato fruits

across 174 tomato accessions (Zhang et al., 2016). Previous attempts to enhance AsA in model plants and several crops such as tomato, potato, and rice indicate that increased AsA content results in higher biomass and greater tolerance to abiotic stresses (Lisko et al., 2013b). Therefore, it is possible to simultaneously improve nutritional content, yield, and stress tolerance by breeding for AsA content.

In spinach, GWAS has been carried out for various traits (Qin et al., 2017, Awika et al., 2019, Awika et al., 2020, Chitwood et al., 2016); however, none have focused on AsA content. In this study, we evaluated the variability of vitamin C content within spinach germplasm and used GWAS to identify molecular markers associated with the trait that could be utilized in breeding programs. Our results indicate that natural variation in vitamin C content exists in spinach germplasm, making it a promising target for research to enhance spinach's nutritional quality.

2.2 Objectives and Hypothesis

i) Screen spinach germplasm to identify accessions with high AsA content.

Hypothesis: There is enough natural variation in AsA content within spinach germplasm to enhance AsA content through traditional breeding approaches.

ii) Identify molecular markers associated with high AsA content in spinach.

Hypothesis: Differential AsA content between accessions is associated with genetic variation in spinach germplasm.

2.3 Materials and Methods

2.3.1 Plant material and growing conditions

A total of 360 spinach accessions were obtained from the USDA-National Plant Germplasm System (NPGS). The USDA-NPGS collection's accessions were grouped by Awika et al. (2019) into regions depending on their reported origin. Germplasm evaluated included 80 accessions from Turkey; 36 from the USA; 21 from Afghanistan; 18 from Macedonia; 16 from China; 15 from Iran; 11 from India; nine from Belgium; six from Syria; five each from Hungary and Japan; four from France; three from the Netherlands, Georgia and Spain; two from Egypt, Ethiopia, Greece, Italy, Pakistan, South Korea, and the United Kingdom; one from Denmark, Germany, Iraq, Mongolia, Nepal, Poland, Serbia, the Soviet Union, Sweden and Taiwan; and 101 accessions of unknown origin.

2.3.2 Growth conditions and experimental design

The 360 spinach accessions were grown under controlled conditions in growth chambers at the Texas A&M AgriLife Research and Extension Center located in Weslaco, Texas. The growth chambers were set to an average temperature of 23 °C, 11 hours of light, and a light intensity of 120 µmol/m^2/s.

The 360 spinach lines were sown in 500 cc pots that contained BM2 germination mix (Berger, Saint-Modeste, QC) supplemented with Osmocote classic 14-14-14 slow-release fertilizer in a proportion of 82:1 v/v. Spinach plants were grown for 2 months with daily watering as needed and fertilized weekly with Water Soluble Tomato Plant

Food (Miracle-Gro, Marysville, OH). Accessions were divided across 24 different trays. Each tray contained three pots of spinach cv Viroflay as a control for normalizing data and 15 different test accessions, for a total of 18 pots per tray. Three biological replicate plants per accession were evaluated.

2.3.3 Tissue collection

Leaf tissue (150 mg) from 2-month-old plants was collected to measure ascorbic acid content. Tissue collection was performed during the morning. Collected leaf tissue was placed in 15 mL centrifuge tubes, flash frozen in liquid nitrogen, and stored at −80 °C until AsA quantification.

2.3.4 Quantification of AsA content

Ascorbic acid content was quantified using a previously described ascorbate oxidase method (Lorence et al., 2004). For each sample, two technical replicates were performed. Tissue was homogenized in a MiniG™ 1600 grinder (Spex®SamplePrep, Metuchen, NJ) for 35 seconds at 1500 rpm, using a pre-cooled aluminum block to avoid tissue thaw. Six premium grade BBs (Daisy®, Rogers, AR) were added to each tube to aid grinding. Immediately after grinding, 2.25 mL of ice-cold 6% metaphosphoric acid was added to each tube, followed by mixing. Then, 0.75 mL of the plant extract was transferred to 1.5 mL conical tubes in duplicate to create the technical replicates. Tubes were centrifuged at 16 g for 5 minutes at 4 °C, and the supernatant was decanted into

new 1.5 mL centrifuge tubes. During the whole process, samples were kept on ice and away from direct light to prevent ascorbic acid oxidation.

Oxidized and reduced ascorbic acid contents in the samples were measured spectrophotometrically at 265 nm absorbance using 10-mm pathlength UV cuvettes (Brand GMBH + CO KG, Wertheim, Germany) and a NanoDrop OneC spectrophotometer (Thermo Fisher Scientific, Waltham, MA). The spectrophotometer was blanked with 1 mL of 0.1 M K-phosphate buffer in disposable UV cuvettes. Samples were prepared by adding 950 µL of K-phosphate buffer + 50 µL of plant extract into new UV cuvettes and initial absorbance was recorded. To quantify oxidized AsA, 1 µL of 1 mM dithiothreitol (DTT) was added and samples were mixed. The UV cuvettes were placed in a dark environment and incubated for 20 min at room temperature before recording the final absorbance. Reduced ascorbate was quantified similarly, but instead of DTT, 1 unit/µL ascorbate oxidase was used.

The total ascorbate content was calculated with the previously reported formula (Lorence et al., 2004):

Total change in absorbance=[Reduced ascorbate]+[Oxidized ascorbate]

$$\text{Total ascorbate content}=\left\{\frac{\left[\frac{(\text{Total change in absorbance})}{14.3}\right]\times 20\times 0.75}{(\text{Sample weight in grams})}\right\}$$

2.3.5 Single nucleotide polymorphism (SNP) markers

SNPs reported by Awika et al. (Awika et al., 2019) were utilized for genome-wide association mapping. SNP markers were obtained by a ddRADseq genotyping-by-

sequencing protocol. A total of 6,167 SNPs were utilized, with the highest number (1458 SNPs) located on chromosome 4 (Chr4) followed by Chr3, Chr1, and Chr2 (1060, 1027, and 1027 SNPs, respectively). Chromosome 6 and 5 had the fewest SNPs, with 838 and 757 SNPs, respectively (Awika et al., 2019). On Chr1 they were located between the position 64515 and 50544189. On Chr2 they were located between the position 34941 and 60452741. On Chr3 they were located between the position 294020 and 112131948. On Ch4 they were located between the position 70156 and 122918674. On Chr5 they were located between the position 83687 and 69232026. On Chr6 they were located between the position 293769 and 46051220.

2.3.6 Population stratification & kinship

Population structure was estimated using the allelic ancestry-based admixture model (Falush et al., 2007, Alexander et al., 2009) in STRUCTURE Version 2.3.4 (Pritchard et al., 2000). The parameters used in STRUCTURE were 10,000 burn-ins with 1,000 replications on the 6,167 SNPs used for the K values 1 to 10. The ideal K value of the population structure (*Q*) was established using the Evanno method (Evanno et al., 2005). Briefly, the ideal cluster was identified by the highest value at the change of K (ΔK). Then within that cluster, the subpopulation with the highest mean value of Ln likelihood was selected. A kinship matrix (k) was created to include the possibility of hidden allele sharing as described by Blouin (2003) under the realized relational matrix (Endelman and Jannink, 2012).

2.3.7 Genome-wide association methods

The best linear unbiased prediction values (BLUPs) were estimated using the software JMP® version 14.0, SAS Institute Inc., Cary, NC (JMP®, 2019). Three linear models were used in the association study; (1) the generalized linear model (GLM); (2) the compressed, mixed, linear model (cMLM, Q + k) on a per trait basis, giving us estimates of genetic and residual variance at the trait level; and (3) the cMLM on a per marker trait basis, giving us estimates of genetic and residual variance at the marker level (Awika et al., 2019). In both cMLMs, the Q was treated as covariate and k as a random coefficient. In the MLMs, compression and "population parameters previously determined" (P3D) were applied. The compression both reduced computing time and increased statistical power, and the P3D did not harm our statistical power while decreasing computing time (Zhang et al., 2010). Association models were run on TASSEL Version 5.2.54 (Bradbury et al., 2007).

2.3.8 Anchoring of putative genes to significant associated SNPs

The putative genes anchored near or on significant associated SNPs were identified using the genomic browser www.spinachbase.org (Collins et al., 2019), which contains the partially sequenced spinach genome. The anchored genes were tabulated individually in the browser by identifying the gene nearest to the position of the SNPs.

2.3.9 Functional enrichment analysis of anchored genes and phylogenetic analysis of high- and low- AsA genotypes

Functional enrichment analysis of the input spinach genes was performed based on the gene ontology (GO) terms and gene families represented. The agriGO (Du et al., 2010) and GenFam (Bedre and Mandadi, 2019) web tools were used to analyze enriched GO and gene family categories using the Fisher exact test. The Benjamini-Hochberg (Benjamini and Hochberg, 1995) approach was used to adjust the *p* values in the GO and GenFam analyses. Phylogenetic analysis of genotypes was performed using TASSEL Version 5.2.54 (Bradbury et al., 2007). A phylogenetic tree was constructed using the neighbor-joining method and visualized using iTOL Version 6 (Letunic and Bork, 2021).

2.4 Results and Discussion

2.4.1 High variation of leaf ascorbic acid content in germplasm indicates good potential for improvement via breeding

We evaluated AsA content in a collection of 352 (8 lines died or did not germinate) spinach accessions that were obtained from the USDA-NPGS and represented a cross-section of spinach genetic diversity. For each accession, we measured three biological replicates (3 pooled plants per replicate) and two technical replicates per biological replicate. The mean of the population's AsA content was 0.3766 µmol/g fresh weight (FW), with a range of 0.02–2.12 µmol/g FW (Figure 2.1, Supplemental Table 1). The coefficient of variation for the population's mean AsA content was 61.58%, indicating high variability between accessions, most likely due to the dioecious nature of spinach.

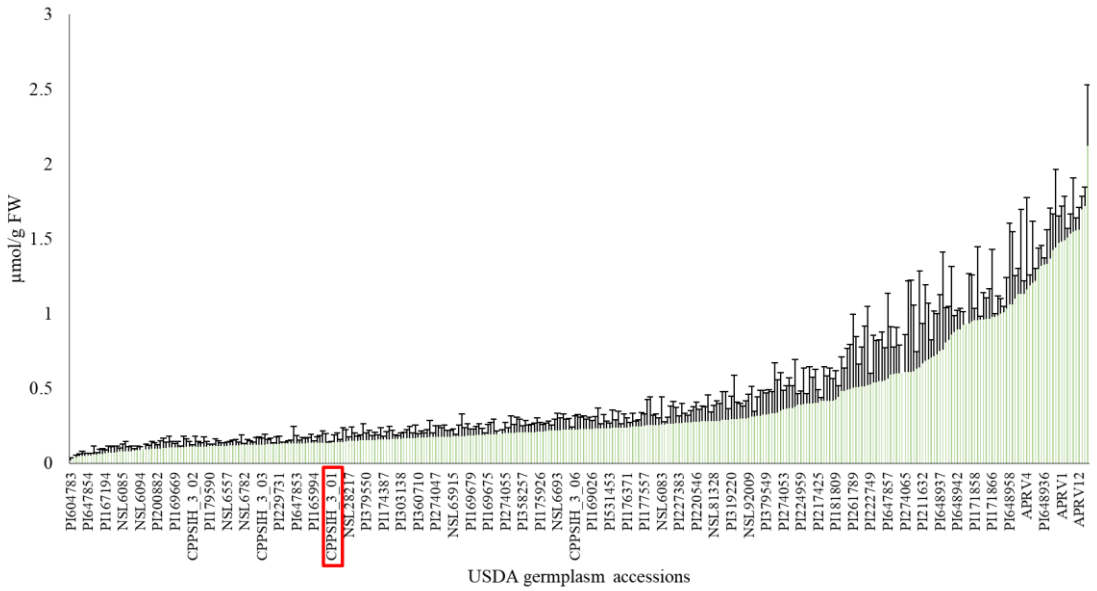

Figure 2.1 Phenotypic diversity of ascorbic acid content in USDA-NPGS spinach germplasm. The red box indicates the commercial cv Viroflay. Due to space limitations, only a few accession IDs are labelled (Mean value +SD, *N* = 3)

The commercial control cultivar Viroflay had an AsA content of 0.1414 µmol/g FW, a value below the population average, which suggests that there is an opportunity to enhance AsA content in commercial varieties of spinach using available genetic diversity.

Similar results have been reported in other crops where diversity of AsA content has been measured. Lisko et al. (2013a) evaluated 24 rice (*Oriza sativa*) accessions from US, Japan, Taiwan, and the Philippines showed a broad spectrum in AsA diversity. Foliar measurements at the V2 developmental stage ranged from 1.5-8 µmol/g FW in various accessions (Lisko et al., 2013a). Likewise, high variability was found when tomato (*Solanum lycopersicum*) germplasm was analyzed for AsA content in the fruit (Bhandari et al., 2016). That study included nine cherry-type and seven non-cherry-type

tomatoes from Korea, as well as 103 accessions from Taiwan, Vietnam, and Korea with 34 cherry-type and 69 non-cherry-type tomatoes. The germplasm collection had a high diversity of AsA levels. The non-cherry type had the larger range, from 0.069-2.203 µmol/g FW, but a large range was also obtained for the cherry type, with values from 0.185-1.847 µmol/g FW. For the commercial cultivars, on the contrary, only cherry-type tomatoes showed a broad diversity in AsA content, having a range of 0.060-1.287 µmol/g FW. Since these cultivars are commercial, intended or unintended selection during breeding has probably contributed to a high variation of AsA content. For the non-cherry type, the range was much smaller, only 0.811-1.069 µmol/g FW (Bhandari et al., 2016).

Other leafy greens have been also screened for AsA content. Llorach et al. (2008) screened for vitamin C content in five lettuce (*Lactuca sativa* L.) varieties and escarole (*Cichorium endivia* var. *crispa*), obtaining AsA contents ranging from 0.1589-1.1066 µmol/g FW (Llorach et al., 2008). In another study of one cultivar each of lettuce, white cabbage (*Brassica oleracea* L. var *capitata* L.), Chinese cabbage (*B. chinensis* L.) and mugwort (*Artemisia vulgaris* Cantley), these crops had AsA measurements of 0.1418 µmol/g FW, 1.0669 µmol/g FW, 1.4358 µmol/g FW and 1.9920 µmol/g FW, respectively (Bahorun et al., 2004). Taken together, our study of spinach and other reports in leafy greens (Llorach et al., 2008, Bahorun et al., 2004), fruits (Bhandari et al., 2016), and grains (Lisko et al., 2013a) indicate there is high natural variation in AsA content between cultivars of many crops, and suggest a potential for improvement.

We grouped the germplasm accessions by geographic origin to determine whether there is a relationship between accession origin and AsA content (Figure 2.2). While statistical differences were observed (Student's t-test, $p < 0.05$) in AsA content between regions, there were no clear geographic trends. However, accessions from Eastern Europe, South Asia, and the US East Coast had more variability in AsA content than accessions from other regions, due largely to a larger maximum AsA content range. It has not been studied yet whether the diversity of AsA content between regions results from unintentional or targeted breeding efforts. This result is consistent with several other spinach studies that found no relationship between phenotype and geographic origin when analyzing traits such as oxalate concentration (Shi et al., 2016b), leafminer resistance (Shi and Mou, 2016), mineral element concentration (Qin et al., 2017), leaf surface texture, petiole color and edge shape (Ma et al., 2016), and bolting, plant height, and leaf erectness (Chitwood et al., 2016).

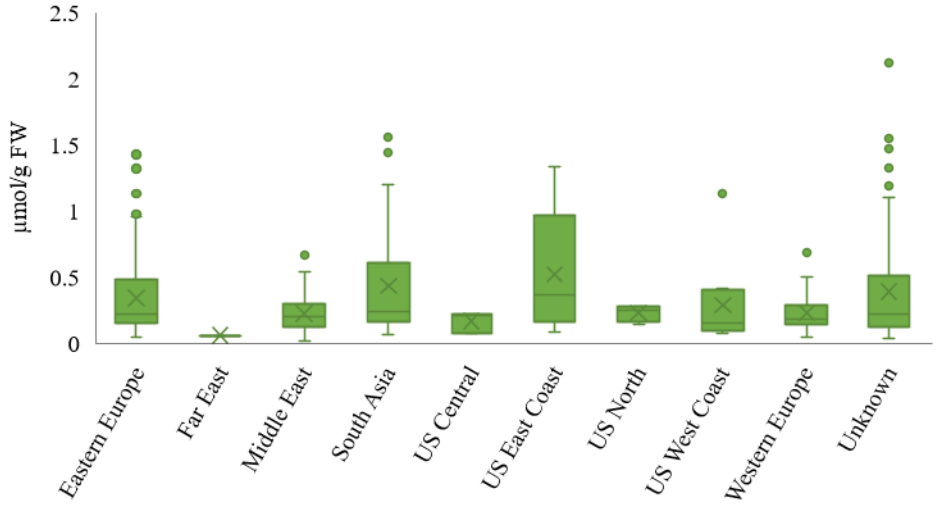

Figure 2.2 Relationship between geographical origin and ascorbic acid content in spinach germplasm. The "X" denotes the region mean, the boxes represent the inter-

quantile range, the line between boxes is the median, and the dots show the outliers outside the region maximum. Different letters indicate statistical differences between regions at $\alpha = 0.05$.

2.4.2 Population structure analysis classifies 270 spinach accessions into two population groups

We chose 270 of the AsA-screened accessions for downstream analysis due to availability of genotypic data. The population structure of the 270 spinach accessions was estimated with the software STRUCTURE version 2.3.4 for K=1–10. Using Structure Harvester (Earl and Vonholdt, 2012), the most likely number of subpopulations that obtained the highest delta K value was K=2 and the second highest was for K=4 (Figure 2.3), with K representing the number of clusters. These results indicate that our spinach accessions are divided into two populations. A membership probability cutoff (Q value) of 0.60 was used to divide the spinach panel into two (Q1 and Q2) main populations, while the remaining accessions showing membership proportions $Q < 0.60$ formed an admixed group (Qm). The population Q1 contains 237 accessions, with the largest number (79 accessions, 33.33%) from East Europe. Population Q2 contains 19 accessions; nine are from South Asia (47.37%). The remaining 14 accessions fell in the admixture group Qm.

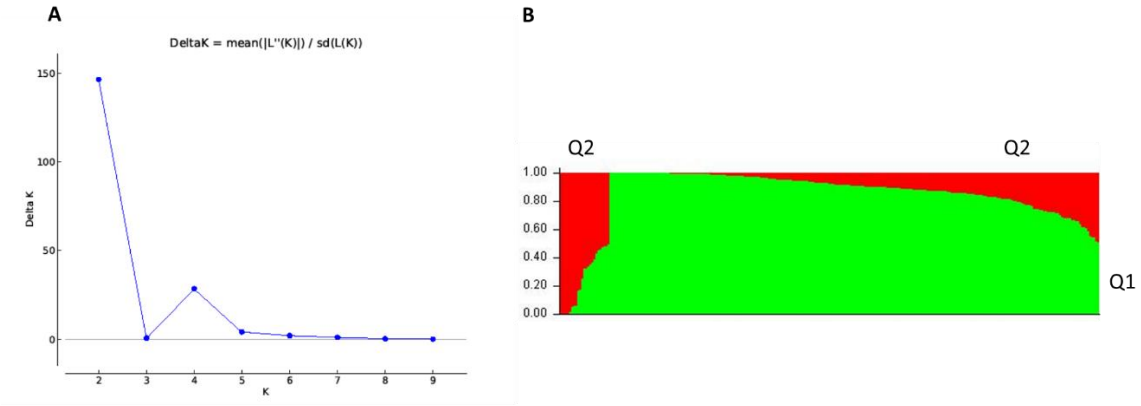

Figure 2.3 Population structure analysis classifies 270 spinach accessions into two population groups based on delta K analysis (A) Likelihood estimate plot showing the number of genetically distinct clusters (K); (B) two populations (Q1 and Q2) estimated from the admixture model (individual accessions on the horizontal axis and the assigned probability on the vertical axis)

A recent study also reported two main populations in spinach when using the same set of SNP markers (Awika et al., 2019). Likewise, another study reported a two-population structure (Q1 and Q2) using the same population of spinach accessions but a different set of SNP markers to perform an association analysis for oxalate concentration (Shi et al., 2016b). However, an association study investigating the mineral concentration in spinach accessions found the data supported four populations within their 292 accessions (Qin et al., 2017).

2.4.3 GWAS models identify unique and overlapping markers associated with ascorbic acid content in spinach

We assessed a collection of 6,167 previously reported genetic markers (Awika et al., 2019) to identify any significantly associated with AsA content. The data were analyzed in TASSEL Version 5.2.54 (Bradbury et al., 2007) using the generalized linear model

(GLM) (Nelder and Wedderburn, 1972), the compressed mixed linear model (cMLM) with the population parameters previously determined (P3D) approach (Zhang et al., 2010), and the perMarker model (Yu et al., 2006). To account for the false discovery rate, we established thresholds for markers significantly associated with AsA content in the three models using the Benjamini-Hochberg (Benjamini and Hochberg, 1995) multiple comparison procedure at $p = 0.05$ (Figure 2.4). The calculated thresholds were 4.44E−04, 3.37E−04, and 2.56E−04 for GLM, P3D, and perMarker, respectively.

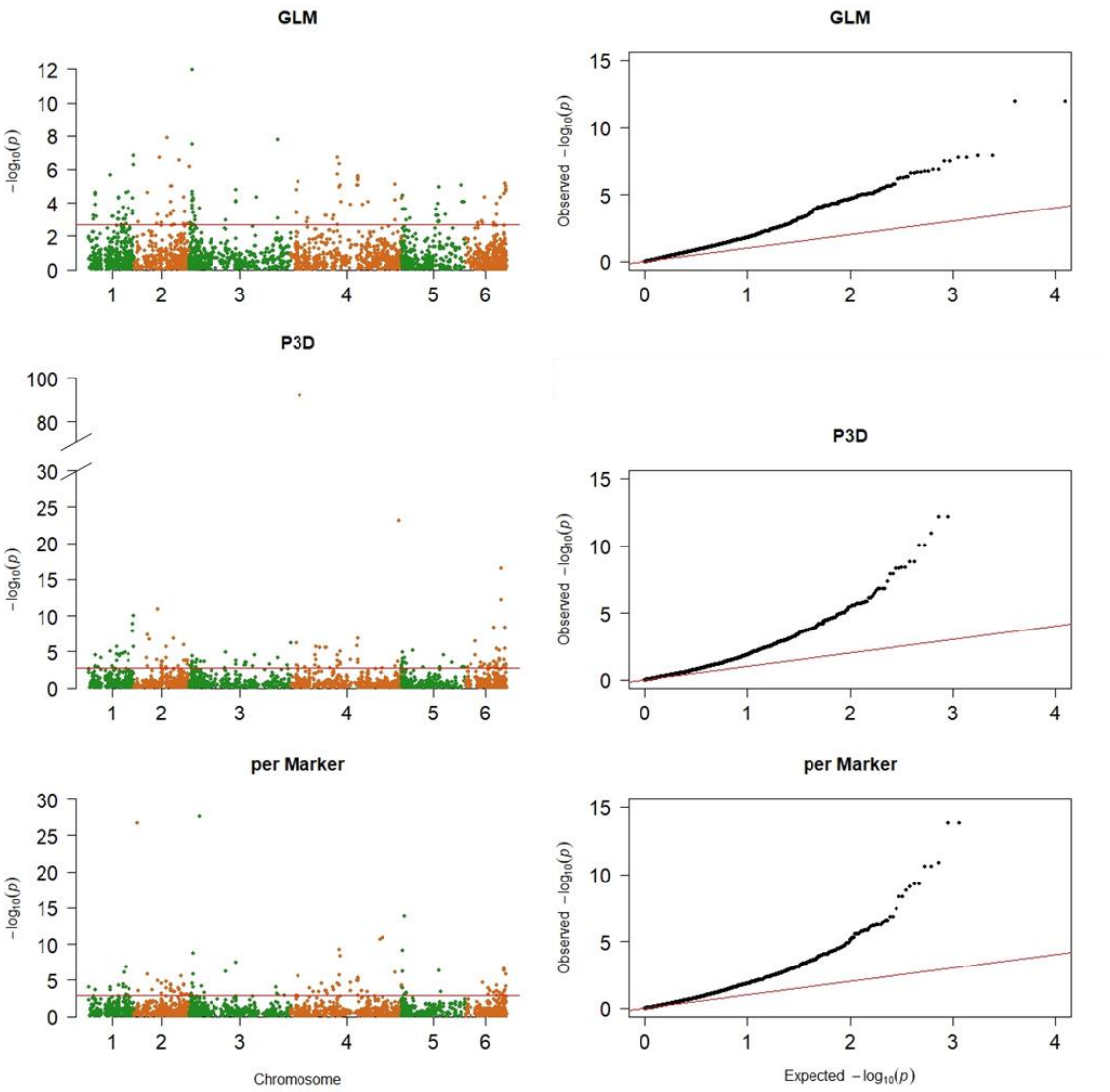

Figure 2.4 SNP markers significantly associated with ascorbic acid content in spinach. Left, Manhattan plots showing threshold and chromosome distribution by association model. Significance thresholds were calculated with Benjamini-Hochberg (Benjamini and Hochberg, 1995) method at $\alpha = 0.05$ (red horizontal lines); Right, Quantile-quantile (QQ) plots showing *P*-value deviations. Generalized linear model (GLM), compressed mixed linear model (cMLM) with Population parameters previously determined (P3D) and cMLM with re-estimation of variance component after each marker (perMarker)

The GLM identified 274 significant markers, the highest number of AsA-associated markers, followed by 204 markers found by the P3D, and 158 markers identified by the perMarker model. A total of 490 SNPs were identified by at least one of the three models (Figure 2.5); however, only 27 SNPs were commonly identified by all three models. An additional 43 SNPs were identified by both GLM and P3D, 43 SNPs were identified by GLM and perMarker, and 10 SNPs were identified by P3D and perMarker. The GLM identified the highest number of unique markers (161 SNPs), as compared with the P3D (124 SNPs) and the perMarker model (78 SNPs) (Supplemental Table 2, Figure 2.5).

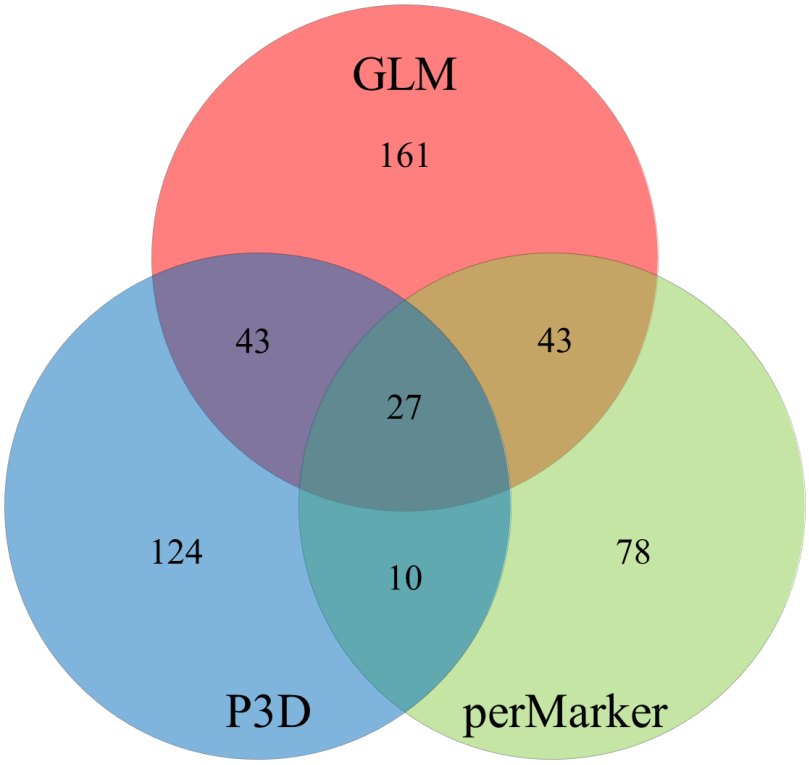

Figure 2.5 Unique and overlapping markers associated with ascorbic acid content in spinach. Generalized linear model (GLM), compressed mixed linear model (cMLM) with population parameters previously determined (P3D) and cMLM with re-estimation of variance component after each marker (perMarker)

Other groups have discussed differences in the number of associated markers identified by models, indicating advantages and disadvantages of each approach (Wang et al., 2014, Zhang et al., 2010, Yu et al., 2006). In association studies, false positives are often the main concern when reporting significantly associated markers, and the GLM can report false positives since population structure and kinship are not simultaneously integrated during the analysis. However, the computing time of the GLM is very low compared to mixed linear models (MLMs) (Wang et al., 2014). Another option is to use the compressed MLM (cMLM) with re-estimation after each marker (perMarker), which reduces the computing time and improves statistical power via compression by clustering individuals into groups. In this model, run time is proportional to the number of groups cubed, rather than the number of individuals. The disadvantage of the perMarker model is that it requires re-estimating variance components after each marker. A final approach used with the cMLM is population parameters previously determined (P3D), which improves computing time but does not affect statistical power (Zhang et al., 2010). Both forms of cMLM have an additional disadvantage when small samples are used, as they tend to produce inaccurate and irrelevant estimations of polygenic factors (Yu et al., 2006).

Many association studies have been performed in spinach and other crops. Shi et al. (2016a) assessed resistance to Stemphylium leaf spot (*Stemphylium botryosum* f. sp. *spinacia*) among 273 spinach accessions. A GLM and MLM, both using Q as population structure, were performed to identify significant SNPs. The GLM identified 14 SNPs associated with pathogen resistance, but the MLM only identified 7 SNPs.

Another association study for root morphology traits in maize also used both a GLM and MLM. A total of 297 inbred maize lines were used for this analysis and several root traits were measured. They report 355 SNPs from the GLM and 28 SNPs from the MLM (Wang et al., 2019). Ma et al. (2016) concluded that the GLM in general can detect more markers associated with traits of interest when compared with the MLM. Our results support this conclusion.

Here, we found 27 SNPs that were significantly associated with AsA levels by all three models (GLM, P3D, and perMarker) (Supplemental Table 2). No major QTL associated with AsA content was detected within commonly associated markers, since all 27 SNPs had low average r^2 values for all models (8% GLM; 13% P3D; 18% perMarker). Some SNPs identified by the perMarker and P3D models have much higher r^2 values. The r^2 of these SNPs might be related to their observed *p*-value, because they all have the lowest *p* values among the markers identified by the respective model. One example of this is the marker 37554_100 which had an r^2 of 87% with the model perMarker but only 8%, and 9% with GLM and P3D, respectively. Other association studies have reported low r^2 values in situations where the trait of interest is probably controlled by a group of several minor genes (Shi et al., 2016a, Wu et al., 2020).

There may be false positive markers identified by our GLM, given that this model is known to have less control over false positives, and our GLM model reported more SNPs significantly associated with AsA than the two other models (P3D and perMarker). Quantile-quantile (QQ) plots (Figure 2.4) can be used to observe power differences between models and control for false positives (Kaler et al., 2020). As

expected, both cMLMs do not have a straight line and are more separated than usual from the expected line. By contrast, the GLM model has a straighter line on the QQ plot than expected, although the separation from the 1:1 line is also present. Despite the two cMLM models having similar QQ plots, only 37 SNPs were associated with AsA content by both models (10 P3D and perMarker only, 27 shared with GLM model). Therefore, both cMLMs are likely not effectively controlling false positives and false negatives. In future work, the use of tree models might be more effective to analyze this type of data, since it could control false positives and avoid declaring false negatives. Despite these caveats, the 27 AsA-associated SNPs markers identified by all three models could be primary targets for validation by researchers working to improve AsA content in spinach.

2.4.4 Markers associated with ascorbic acid content are dispersed throughout the genome

The 490 significant, AsA-associated SNPs we identified were distributed along the six chromosomes of the spinach genome. Of those markers, we found an almost even distribution across Chr1, Chr2, Chr3, Chr4 and Chr6–17.76%, 18.57%, 17.96%, 18.37% and 17.35%, respectively. Chr5 had fewer markers than the other chromosomes, only 10% of identified SNPs (Figure 2.6). The distribution of significant SNPs across the whole spinach genome could be related with the presence of four different biosynthetic pathways for AsA (Suza et al., 2010).

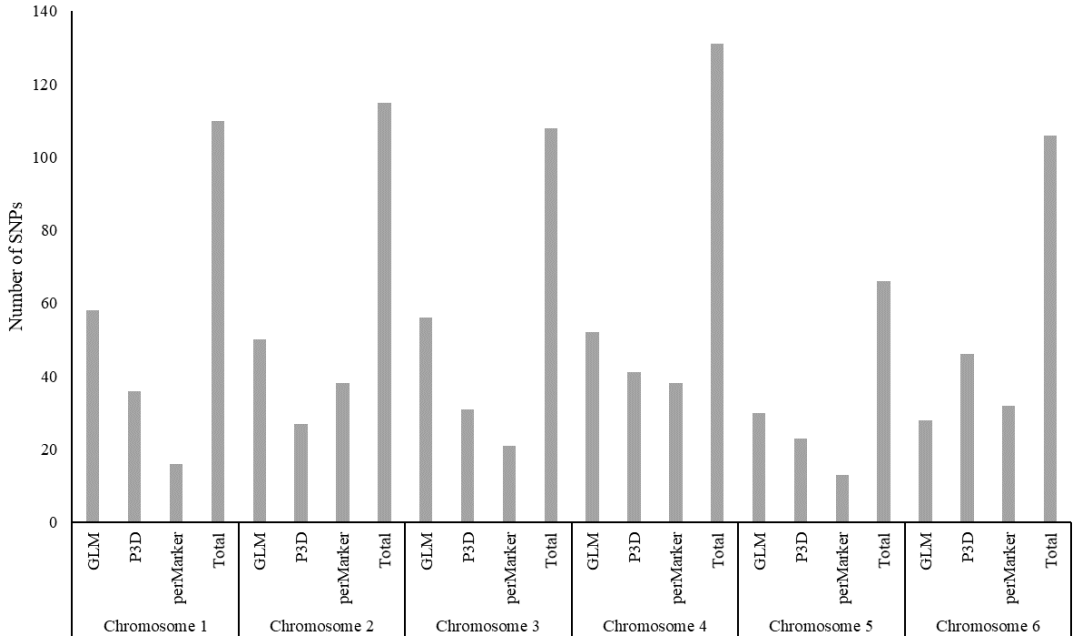

Figure 2.6 Number of ascorbic acid content–associated SNPs distributed across the spinach genome

When AsA quantification and a subsequent association study was performed in tomato fruits, the authors found five significant SNPs among four chromosomes (Sauvage et al., 2014). Genes that had been previously reported to be involved in ascorbic acid biosynthesis were mapped in introgression lines of tomato and were found to be spread across 9 chromosomes (Zou et al., 2006). Furthermore, the *VTC* genes, which are directly involved in AsA biosynthesis, were mapped in *Arabidopsis thaliana* mutants. Four *VTC* genes, VTC1, VTC2, VTC3, and VTC4, were mapped onto Chromosome 2, 4, 2, and 3, respectively (Conklin et al., 2000). Therefore, although other studies have identified less SNPs associated with AsA, they were also found on several chromosomes in agreement with our results.

2.4.5 Anchored genes show a wide range of functions with putative roles in biosynthesis processes and response to stress

We anchored significant SNPs from our analysis to putative genes by looking up SNP position in the spinach genome using the webpage www.spinachbase.org. Using the 490 significant SNPs from the three association models, we were able to identify 310 candidate genes. Some genes had more than one significant marker. For example, three markers identified by the GLM model (*20248_143, 20250_0 and 34990_9*) were anchored within the same gene. In total, 113 unique markers that were identified by the GLM model fell within gene sequences, and an additional 51 markers were within 146 kb of a gene. When analyzing SNPs from the P3D model, 95 unique markers fell within the sequence of a gene, and 29 further unique markers were within 51.23 kb of an annotated gene. Finally, when using the perMarker method, 57 unique markers were anchored within a gene sequence, and 22 markers were within 180.62 kb of a gene (Supplemental Table 3).

We also anchored markers that were identified by multiple modelling methods. Out of the 43 SNPs identified by both the GLM and P3D models, 30 markers were within genes, and 13 markers were within 37.7 kb of a gene. Similarly, of the 43 SNPs identified by both the GLM and perMarker models, 33 markers were within a gene sequence, and the other 10 markers were within a distance of 191.85 kb of a gene. All 10 markers identified by both the P3D and perMarker methods were within a gene sequence. Finally, 12 of the SNPs identified by all three models were found within a

gene, and the remaining 15 markers were within 191.64 kb of a gene (Supplemental File 2).

Out of the 310 marker-anchored, reported genes, 291 genes encode known proteins (Supplemental Table 3). Notably, our analysis did not identify any genes that are directly involved in AsA biosynthetic pathways. Therefore, we hypothesize that the variability in AsA accumulation that we observed across spinach accessions may be due to substrate availability or plant hormone regulation rather than differences in AsA biosynthetic enzyme activity. Several pathways are involved in AsA synthesis (Suza et al., 2010); therefore, the genes we identified may limit or boost AsA synthesis at multiple entry points or in multiple pathways. In addition, plant hormone regulation may also be important to coordinate AsA content with other plant defense systems. Future work will aim to test these hypotheses.

Ascorbic acid has many different functions in plants. It can work as an antioxidant in scavenging ROS, it is part of many metabolic processes as an enzyme cofactor, and it is part of several signaling pathways including those that control the flowering period, responses to active pathogen activity and the expression of metabolic genes. Therefore, anchored genes were grouped by biological process, molecular function, and putative gene family using the agriGO (Du et al., 2010) and GenFam (Bedre and Mandadi, 2019) web tools, which use the Fisher exact test to analyze enriched GO and gene family categories.

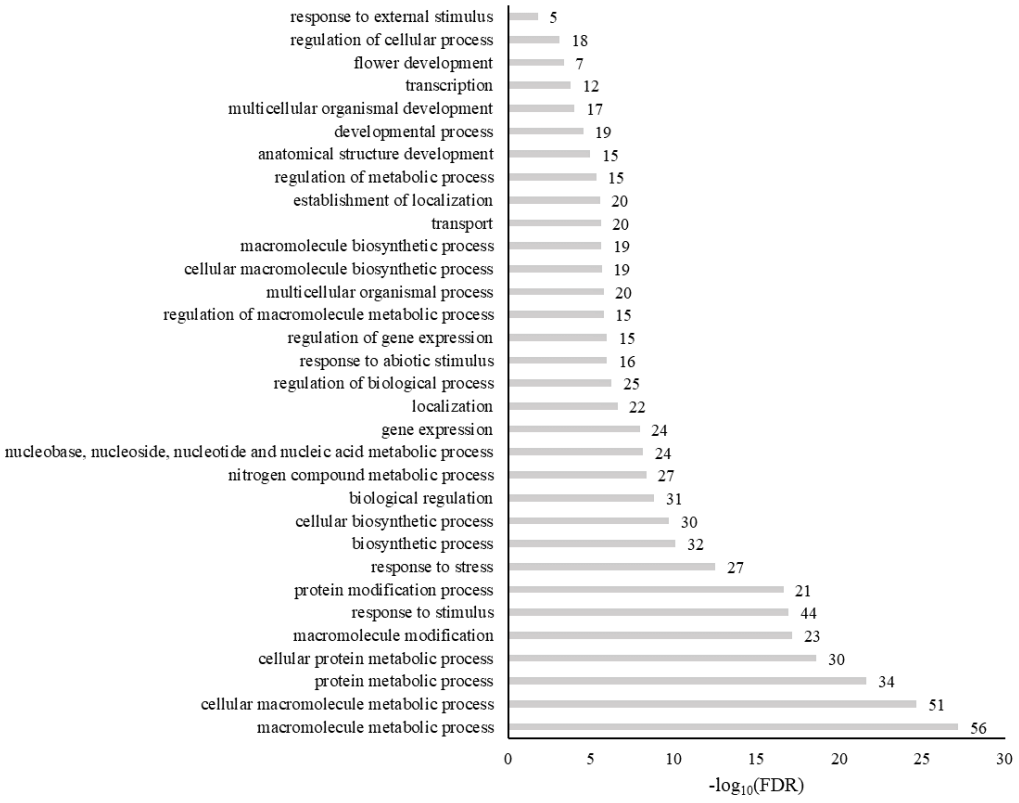

Figure 2.7 Enrichment of gene ontology classification of genes anchored to spinach ascorbic acid content–associated markers by biological process. Numbers next to bars are the genes related to that process

The biological processes enriched in these genes included metabolic processes, responses to a stimulus, responses to stress, biosynthetic processes, and biological regulation (Figure 2.7). When genes were grouped by molecular functions, SNP-anchored genes are enriched for transcription factor activity, protein binding, transferase activity, and transporter activity (Figure 2.8).

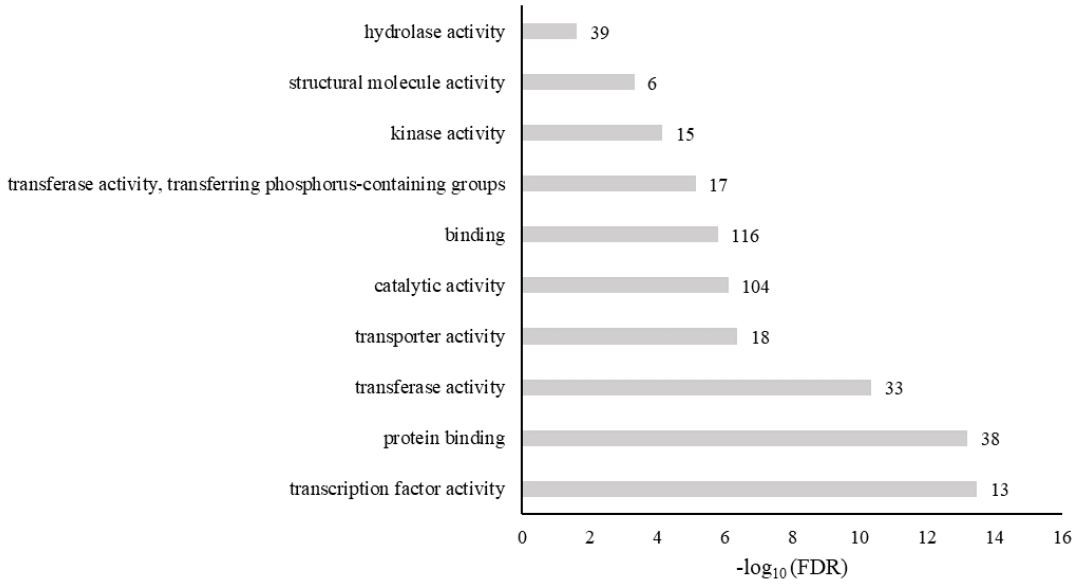

Figure 2.8 Enrichment of gene ontology classification of genes anchored to spinach ascorbic acid content–associated markers by molecular function. Numbers next to bars are the genes related to that function

To narrow down the genes most closely related to AsA synthesis in spinach, a GenFam analysis (Bedre and Mandadi, 2019) for gene family discovery was performed. Four gene families were significant ($p < 0.05$): the RNA helicase gene family, the RING finger domain gene family, the IQD (IQ67-domain) gene family, and the tubulin gene family (Figure 2.9).

The RING finger domain gene family was previously linked with AsA content in Arabidopsis. Wild type and *vtc* mutant Arabidopsis leaves were treated with L-Galactonolactone (L-GalL), the precursor to AsA in the D-mannose/L-galactose pathway (Wheeler et al., 1998). After treatment and exposure to light, the transcription level of the RING finger domain gene *At1g22500* increased (Gao et al., 2011). Furthermore, the authors created transgenic Arabidopsis carrying a luciferase reporter

for the gene *At1g22500*, a C3HC4-type RING finger gene, and tested luminescence after supplementing with L-GalL. Relative luminescence of the reporter was significantly higher after the treatment with L-GalL in comparison to the control (Gao et al., 2011).

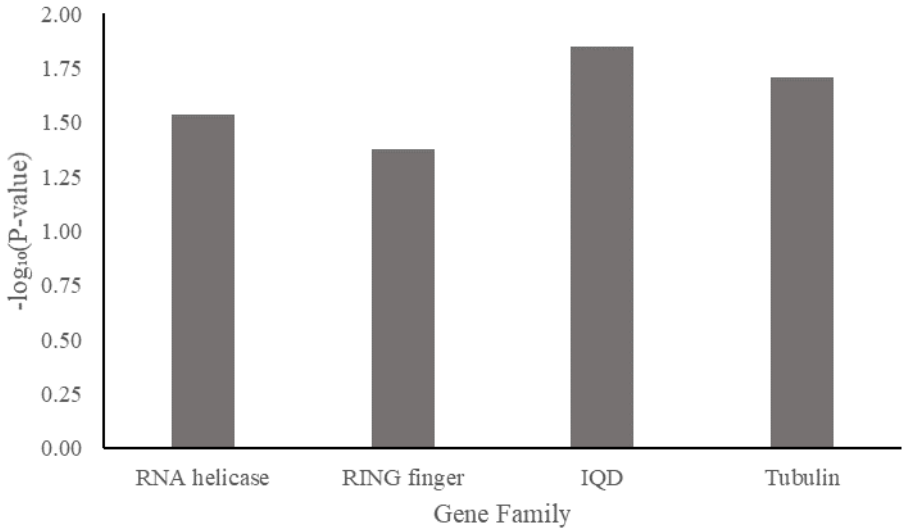

Figure 2.9 Overrepresented gene families among spinach ascorbic acid content–associated genes

RING finger family genes also ameliorate abiotic and biotic stresses and induce hormone signaling, including abscisic acid (ABA) signaling (Dreher and Callis, 2007). Yang et al. (2019) performed an analysis of the RING finger gene family in tomato (*Solanum lycopersicum*), and identified several RING genes that were upregulated with the presence of salt stress, ABA treatment, and drought stress. The relationship between ABA and AsA biosynthesis was further studied in Arabidopsis (Zhang et al., 2020). Plants with a mutation in the *PTP-like Nucleotidase (PTPN*) gene demonstrated increased AsA content in response to a drought stress that would also increase ABA content. It is hypothesized that, evolutionarily, increasing needs for ABA synthesis in

plants drove higher expression levels of *PTPN*, which then produced increased endogenous, inorganic phosphate hydrolyzing GDP/GMP/dGMP/IMP/dIMP, which allowed VTC2 activity to increase AsA content. VTC2 is involved in the AsA pathway (Zhang et al., 2020).

In particular, two genes that encode RING/U-box superfamily proteins, *Spo05860* and *Spo28008*, were associated with 3 and 2 significant SNPs in our analysis, respectively. RING/U-box superfamily proteins are part of the plant ubiquitin proteasome pathway and help to regulate levels of the hormones, gibberellin and ABA. Furthermore, these proteins are involved in the response to abiotic stresses, like salinity, cold, and drought (Sharma et al., 2012).

In addition to genes linked to the enriched GO terms, genes in non-overrepresented processes may also be involved in AsA content. Likely candidates include the genes *Spo11398* and *Spo2944*, which had 4 and 3 anchored SNPs, respectively, and encode cytochrome P450 proteins. These proteins are mainly located in the mitochondria in plants. Several metabolic functions are attributed to them, such as participating in plant hormone catabolism, fatty acid oxidation, and antioxidant synthesis (Werck-Reichhart and Feyereisen, 2000).

Marker *27548_30* (Chr3) was anchored at an exon of the Myb transcription factor *Spo04438*. A study performed using the Chinese-native Birchleaf tree (*Pyrus betulaefolia*), revealed PbrMYB5 was able to bind the dehydroascorbate reductase promoter, which is involved in the recycling process for plant AsA regeneration (Xing et

al., 2019). The authors also demonstrated that overexpressing *PbrMYB5* enhanced AsA levels in transgenic tobacco (Xing et al., 2019).

Another study in Birchleaf discovered the gene *PbrWRKY53,* which is part of the WRKY transcription factor family (Liu et al., 2019). These transcription factors enhance drought tolerance for transgenic tobacco plants by causing an improved water status, a reduction in ROS, and a higher AsA level. Our study found a SNP anchored in the gene *Spo02295,* which is annotated as the probable WRKY21 transcription factor (Liu et al., 2019).

The gene *Spo15092* identified in our study has a functional annotation for an ethylene-responsive transcription factor (ERF). In Chinese cabbage (*Brassica rapa* ssp. *chinensis*) AsA levels increased after the gene *BcERF070* was overexpressed, suggesting that the transcription factor may regulate genes involved in the AsA pathway (Yuan et al., 2020).

Finally, the marker *34444_0,* which was identified by all three models, was anchored in *Spo24572,* with the functional annotation "Salt overly sensitive 1" (SOS1) (Supplemental Table 3). The SOS1 gene was upregulated in Arabidopsis when a salt stress was applied (Shi et al., 2000) and salt stress tolerance was increased in plants overexpressing this gene (Yang et al., 2009). This gene is an important part of the SOS pathway, especially in roots, since it can regulate the amount of Na+ present in root cells (Ji et al., 2013). In conclusion several genes presented have been proven in other studies to be related with AsA synthesis in plants.

2.4.6 The relationship between high- and low-ascorbic acid content spinach accessions tested using the 490 significant polymorphic variants

We performed a family relatedness test using the 15 highest and 15 lowest AsA content accessions and the 490 significant polymorphic variants. A phylogenetic tree was made from 30 accessions based on the neighbor-joining method (Saitou and Nei, 1987) (Figure 2.10).

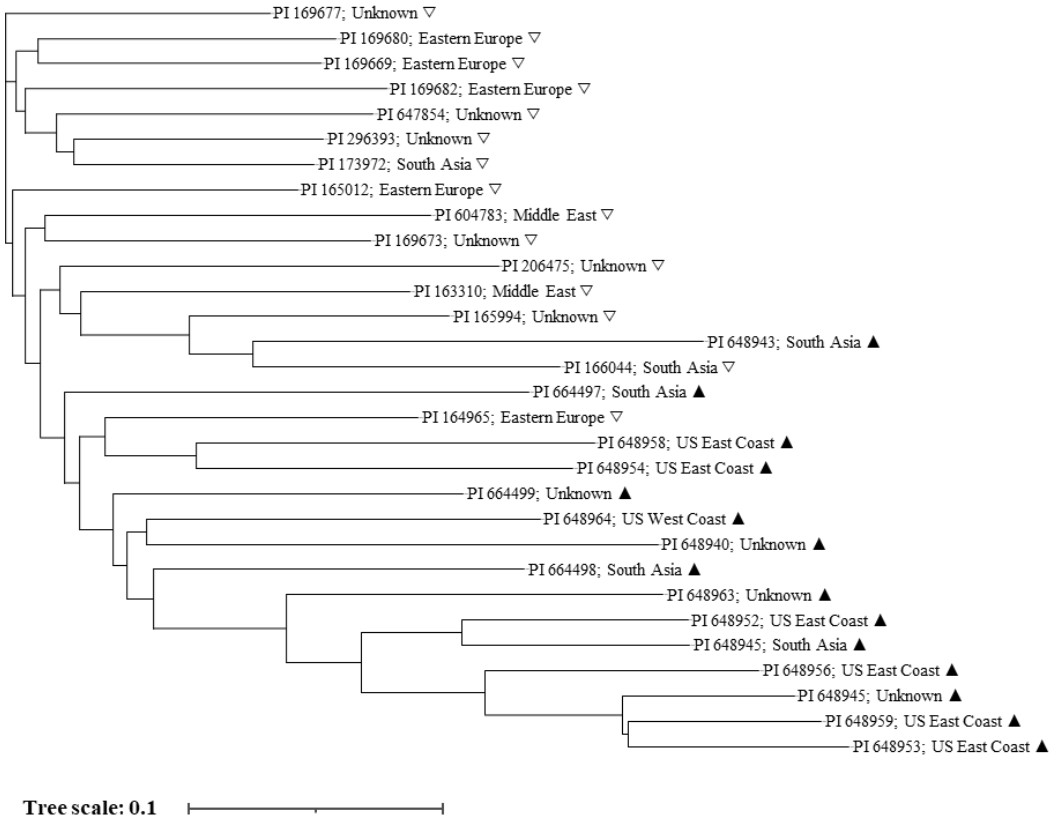

Figure 2.10 Ancestry relationships of spinach accessions with respect to 490 significant polymorphic sites from GWAS analysis. The ancestry history was inferred using the neighbor-joining method. Next to each branch is the corresponding accession identifier, origin and AsA content (low = ▽; high = ▲). Analyses were conducted in TASSEL Version 5.2.54

We found three main, divergent clusters within the tree when sorting the high and low AsA content accessions. Seven accessions, all with low AsA content, make up the two first clades. The third clade contained a shared ancestry, with the remaining eight low AsA content accessions and all 15 high AsA content ones. Within this third clade, with the exception of one accession (PI 164965), only high AsA content accessions are found. This suggests that a portion of SNPs specific to accessions with high AsA content are associated with this trait and upon validation, could be used as part of a molecular breeding selection strategy.

Most of the high AsA content accessions in the phylogenetic tree are of US origin, suggesting unintentional or intentional breeding selection for high AsA content has been performed. The high AsA content accessions PI 648953, PI 648959, and PI 648945,along with the low AsA content accessions present in the first two hereditary branches, could be used as parents to create segregating populations for AsA content marker validation.

Conclusions

Ascorbic acid (AsA) is an important antioxidant with multiple functions in plants and is an essential nutrient in the human diet. Therefore, efforts have been made in many crops to enhance AsA content. Here, we report that the variability of AsA content in spinach germplasm is high, and existing accessions can be utilized for cultivar improvement. One strategy to increase selection efficiency is to use molecular breeding approaches after identifying markers associated with the trait. In this study, we implemented three

genome-wide association models. Each method identified a collection of unique but also overlapping markers, suggesting that a combination of methods can help to narrow down associated polymorphisms in spinach. The 27 SNPs commonly identified by all three models could be used as primarily target for marker validation. Since AsA biosynthesis can occur in plants via several pathways, we expected to find several significant SNPs across all spinach chromosomes. However, we did not identify an association between known AsA biosynthesis genes and AsA content in this panel, suggesting that the differences in AsA content may be related to substrate availability, the effect of plant hormones, or changes to transcriptional regulation. GO- and family enrichment analysis of the genes related to AsA content identified genes involved in metabolic and biosynthetic processes, responses to stimuli and stress, and plant defense signaling. Further work is needed to validate the usefulness of the identified markers for molecular breeding programs, but *in silico* analysis suggests that the identified SNPs differentiate between accession clusters differing in AsA content.

CHAPTER III

EFFECT OF ASCORBIC ACID CONTENT ON SPINACH SALT STRESS TOLERANCE

3.1 Introduction

Crop plants are exposed to numerous threats during their lifespan; beginning the moment the seed is placed into the soil and ending when the crop is harvested. A large portion of these threats are abiotic stresses caused by environmental factors such as drought, flooding, excessive heat, prolonged frost, nutrient deficiency, natural disasters, and saline soils (Nagarajan and Nagarajan, 2010). These stresses can affect plants in several ways and are ultimately detrimental for growth and yield (Singh et al., 2010, Cakmak, 2005). In response, plants have developed several defense or tolerance mechanisms to offset these stresses; for example, compartmentalization, exclusion, or absorption of ions in the cell to modify osmotic pressure, creation and storage of organic solutes, modifications of the layout of cell membranes, and regulation of defense hormones produced by the plant (Pardo-Hernández et al., 2020).

One major, common threat to many crops is stress resulting from high salt concentration in the soil, resulting in soil electrical conductivity higher than ~4 dS m^{-1} (Majeed and Muhammad, 2019). Some of the negative effects of periodic high salinity on plants include reduction in photosynthetic efficiency (Mathur et al., 2013), reduced water intake (Khataar et al., 2018), reduced plant growth (Rahneshan et al., 2018), ion imbalance (Amirul Alam et al., 2015), and low stomatal conductance (Kusvuran, 2012).

Spinach (*Spinacia oleracea*) is categorized as a salt-sensitive crop, with a threshold of 2.0 dS m^{-1} in soil and 1.3 dS m^{-1} for irrigation water (Machado and Serralheiro, 2017). Therefore, spinach yield and quality can be affected by salt stress even when a relatively low salt concentration is present in the soil (Ors and Suarez, 2017, Xu and Mou, 2016). The application of exogenous nutrients like phosphorus and potassium can ameliorate the negative effects on growth and physiological development produced by high salinity in spinach (Kaya et al., 2001). Despite being a salt-sensitive crop, spinach produces several phenolic compounds that can increase its antioxidant capacity and therefore contribute to salt tolerance (Bergman et al., 2001, Howard et al., 2002). In fact, spinach leaves are considered to have high antioxidant capacity, with a score of 17 in oxygen radical absorbance capacity (ORAC) (Cao et al., 1996), and are believed to help humans in diminishing oxidative stress and fight cancer (Pandjaitan et al., 2005).

Because of its high antioxidant capacity and its involvement as an enzyme co-factor (Khan et al., 2011), special attention has been given to the organic compound L-ascorbic acid (AsA). This compound, also commonly known as vitamin C, is a nutrient required by humans that must be taken up predominately from plants (Du et al., 2012). The principal chemical property of AsA is its ability to donate electrons, helping to counteract ROS, such as superoxide, produced during stress and protecting cells from damage (Smirnoff, 2018). Therefore, several strategies have been attempted to improve the AsA content and it effects in plants. For example, exogenous application of ascorbic acid increased salt tolerance in crops such as wheat (*Triticum durum* Desf. var. Waha)

(Azzedine et al., 2011), rapeseed (*Brassica rapa*) (Kaur and Mittal, 2018), maize (*Zea mays*) (Billah et al., 2017), and tomato (*Solanum lycopersicum*) (El et al., 2013). Alternatively, several studies have increased the AsA content in plants by overexpressing AsA biosynthesis or recirculation enzyme genes (Chen et al., 2003, Lorence et al., 2004, Upadhyaya et al., 2011, Xiao et al., 2015, Cronje et al., 2012, Qin et al., 2011). Other studies have reported that increasing the AsA content using genetic engineering approaches has resulted in increased salt tolerance in *Arabidopsis thaliana* (Zhang et al., 2012, Zhu et al., 2014), tobacco (*Nicotiana tabacum*) (Eltelib et al., 2012), tomato (Li et al., 2012b), and rice (*Oryza sativa*) (Wang et al., 2018).

As an alternative approach, the high natural variation of AsA content among spinach germplasm (Chapter 2) can be utilized to improve tolerance to salt stress. In this study, we determined the optimal salt concentration to screen spinach germplasm for salt tolerance under controlled conditions and evaluated the effect of high and low endogenous AsA content on salinity tolerance. Our results indicate that spinach plants with high AsA content can better tolerate salt stress by maintaining photosynthesis and respiration, pointing to the possibility of improving nutritional content and stress tolerance by breeding for AsA content.

3.2 Objectives and hypothesis

i) Determine optimal salt concentration to evaluate salt tolerance in spinach germplasm

Hypothesis: Tolerance and susceptibility to salt stress is dependent on substrate salt concentration.

ii) Determine the effect of ascorbic acid content on salinity tolerance.

Hypothesis: Enhancing AsA content in spinach will result in nutrient-dense cultivars tolerant to salinity stress.

3.3 Material & Methods

3.3.1 Plant material and growing conditions

The commercial spinach (*Spinacia oleracea*) cv. Viroflay and the 5 accessions each of high- and low-AsA-content groups (Chapter II) from the USDA-National Plant Germplasm System (NPGS) were sown in 50-well trays containing BM2 germination mix (Berger, Saint-Modeste, QC) supplemented with Osmocote classic 14-14-14 (slow-release fertilizer in a proportion of 82:1 v/v). Each well had 1 seedling, which was grown for 5 weeks, watered every day, and fertilized with water-soluble Tomato Plant Food (Miracle-Gro, Marysville, OH) each week. Five replications per accession were performed in a completely randomized design. Growth conditions were same as in Chapter 2.

3.3.2 Tissue collection

Leaf tissue (150 mg) from 2-month-old plants was collected to measure the AsA content. Tissue collection was performed during the morning only to reduce variability in AsA content during the day. The collected leaf tissue was placed in 15-mL centrifuge tubes, flash frozen in liquid nitrogen and stored at −80°C until AsA quantification.

3.3.3 Quantification of AsA content

AsA content was quantified using the ascorbate oxidase method described by Lorence et al. (2004) (Lorence et al., 2004) and adapted for the 96-well format. For each sample, two technical replications were performed.

Tissue was homogenized in a MiniG 1600 grinder (SpexSamplePrep, Metuchen, NJ) for 35 seconds at 1500 rpm in a -80°C pre-cooled aluminum block avoiding tissue thaw. Each tube containing tissue had 6 premium-grade BBs (Daisy, Rogers, AR) for grinding aid. Immediately after grinding, 2.25 mL of ice-cold 6% metaphosphoric acid was added to each tube and mixed. Then, 0.75 mL of each plant extract was transferred to 1.5 mL conical tubes. Tubes were centrifuged at 16 g for 20 minutes at 4°C and 0.1 mL of each supernatant was transferred into 0.2 mL VWR PCR tubes with flat caps (VWR International LLC, Radnor, PA). During the whole process, tubes were kept on ice and direct light was avoided to prevent AsA oxidation.

Oxidized and reduced AsA contents in the samples were measured spectrophotometrically at 265 nm absorbance in a 96-well UV-Star Microplate (Greiner Bio-One GmbH, Frickenhausen, Germany) using an Epoch BioTek plate reader (BioTek Instruments Inc, Winooski, VT). The plate was blanked with 0.3 mL of 0.1 M K-phosphate buffer in the 96-well microplate. Samples were loaded on the 96-well microplate by adding 15 µL of sample supernatant followed by 275 µL of 0.1 M K-phosphate buffer and initial absorbance was recorded. Then, 10 µL of 1.2 mM dithiothreitol (DTT) was added to half of the samples on the 96-well plate and 10 µL of ascorbate oxidase (50 U/mL) was added to the other half of the samples to measure the

content of oxidized and reduced AsA, respectively. Every sample was then well mixed and placed in the dark for 20 min at room temperature before recording the final absorbance.

Each day samples were processed a standard curve was calculated using purified AsA (Avantor Performance Materials LLC, Center Valley, PA) dissolved in 0.1 M K-phosphate buffer. For the standard curve, 0.3 mL duplicates of 200, 100, 50, 25, and 12.5 µM AsA were pipetted into the 96-well microplate and absorbance was measured at 265 nm.

Each 96-well microplate contained the 5 different AsA dilutions, 6 samples of 0.3 mL blanks, and a total of 20 extracted samples each with 2 technical replicates for DTT and 20 extracted samples each with 2 technical replicates for ascorbate oxidase. The total ascorbate content was calculated with the formula as reported by Lorence et al. (2004) (Lorence et al., 2004):

Total change in absorbance = [Reduced ascorbate] + [Oxidized ascorbate]

$$\text{Total ascorbate content} = \left\{ \frac{\left[\frac{(\text{Total change in absorbance})}{14.3} \right] \times 20 \times 0.75}{(\text{Sample weight in grams})} \right\}$$

3.3.4 Determination of the optimal salt content for screening for salt tolerance

We determined the optimal salt concentration for screening for salt tolerance by exposing the commercial cultivar Viroflay to seven salt concentrations and a no-salt control. The salt concentrations evaluated were 50, 100, 150, 200, 250, 300, and 350 mM NaCl. Treatments in doses of 10 mL were applied for two weeks. Stomatal conductance (mmol/m²·s) and chlorophyll fluorescence (ratio of variable fluorescence to

maximal fluorescence, Fv/Fm) were measured with a SC-1 Leaf Porometer (Meter, Pullman, WA) and a Chlorophyll Fluorometer OS30p+ (OPTI-SCIENCES, Hudson, NH), respectively. After two weeks, fresh and dry weight was measured to calculate the dry matter content.

3.3.5 Evaluation of the effect of high and low AsA on salt stress tolerance in spinach

Selected spinach accessions at the top and bottom of the AsA content distribution were evaluated for salt tolerance at 0 and 150 mM NaCl. Five replications per treatment combination were performed. Treatments were applied as described in section 3.3.4. In addition, total AsA content was measured at 14 DOT.

3.4 Results and Discussion

3.4.1 Determination of the optimal salt content for germplasm evaluation

We evaluated the spinach cultivar Viroflay for its response to salt treatment from 50 to 350 mM NaCl in 50 mM increments. In addition, a no-salt treatment was added as a control. Stomatal conductance and chlorophyll fluorescence were measured every two days as indicators of plant stress, while fresh and dry weight were evaluated at 14 days to evaluate the effect on yield.

Statistical differences (Students t-test, $p < 0.05$) in stomatal conductance were observed as early as 1 day after NaCl was applied in high concentrations (300–350 mM, Figure 3.1). Then, after 3 days, all NaCl treatments showed statistical differences (Students t-test, $p < 0.05$) as compared to the control until 10 days. Application of 150 mM NaCl showed an intermediate decrease on stomatal conductance, while the

maximum decrease was observed at 300–350 mM. On the other hand, a significant reduction in chlorophyll fluorescence was observed when 300–350 mM NaCl was applied. No significant differences (Students t-test, $p < 0.05$) in chlorophyll fluorescence were detected between treatments from 50 to 200 mM NaCl as compared to the no-salt control (Figure 3.1).

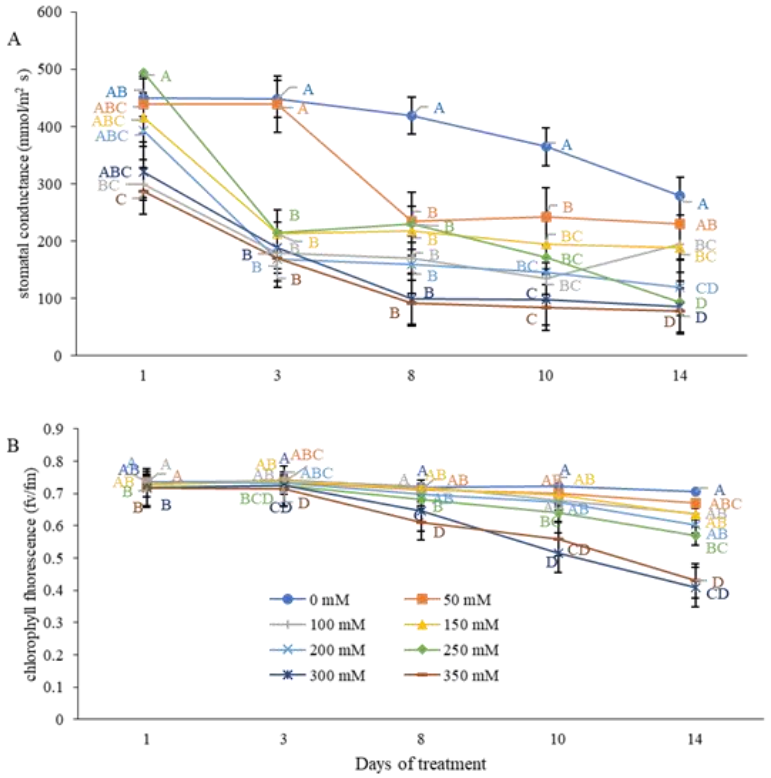

Figure 3.1 Stomatal conductance (A) and chlorophyll fluorescence (B) in response to the different salt concentrations in spinach cv. Viroflay (Mean ±SD, *N* = 10). mM, millimolar. Different letters indicate statistical differences between treatments at α = 0.05.

In addition to stomatal conductance and chlorophyll fluorescence as measures of plant stress, we evaluated the effect of salt stress on yield. The 50 mM NaCl treatment

resulted in a statistically significant increase (Students t-test, $p < 0.05$) in fresh weight of spinach leaves (35.83%), while the highest concentration tested (350 mM) resulted in a decrease of 30.46% in leaf fresh weight as compared to the no-salt control. Intermediate NaCl concentrations (100–300 mM) did not show statistical differences (Students t-test, $p < 0.05$), although the trend shows that fresh weight was reduced as salt concentration increased (Figure 3.2).

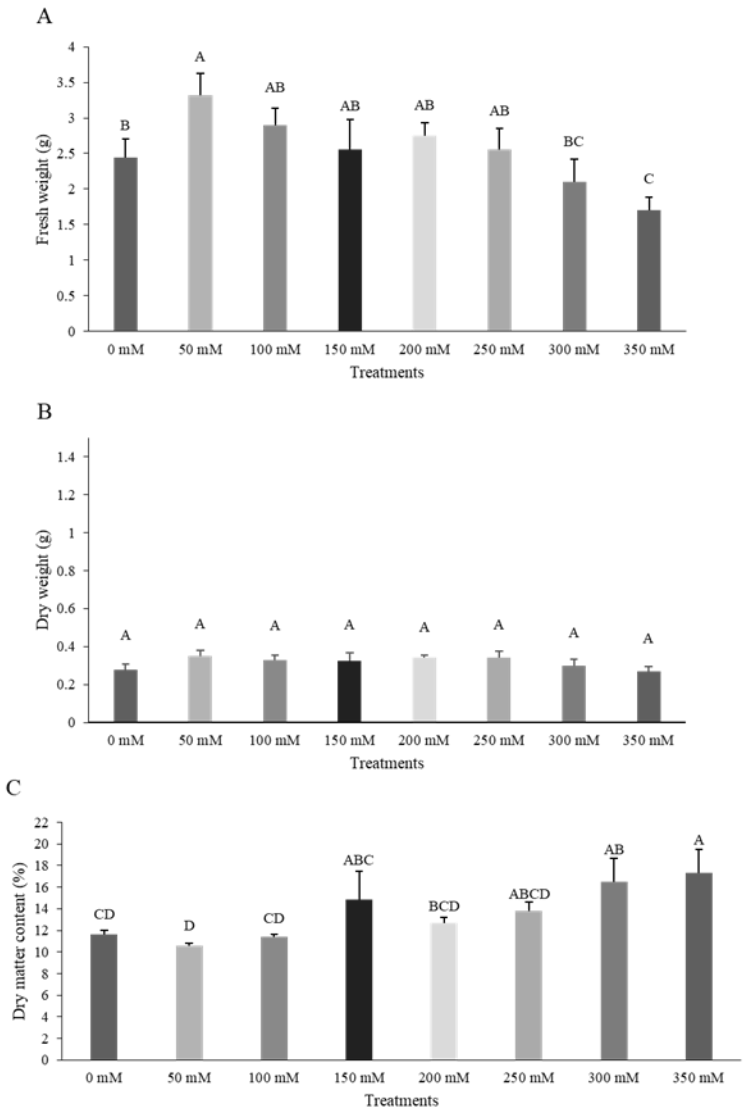

Figure 3.2 Effect of NaCl concentration on plant yield. (A) fresh weight, (B) dry weight, and (C) percent dry matter content in Spinach cv. Viroflay (Mean ±SD, N = 10). mM, millimolar. Different letters indicate statistical differences between treatments at $\alpha = 0.05$.

In addition, the percent dry matter content was higher at 300 and 350 mM NaCl as compared to the no-salt control, reflecting lower water uptake in salt-stressed plants, since the dry weight content did not differ between salt treatments (Figure 3.2B).

Together, these results indicate that spinach can be screened for tolerance at 150 mM and 300 mM NaCl to reflect medium and high salt stress conditions, respectively, since we observed statistical differences (Students t-test, $p < 0.05$) in plant stress and yield variables at these two concentrations.

3.4.2 Effect of spinach AsA concentration on salt stress tolerance

3.4.2.1 Selection of spinach lines for evaluation of salt tolerance

To evaluate the effect of AsA on salt stress tolerance, we selected five high AsA content and five low-AsA-content lines based on preliminary germplasm screening (Chapter II). Here, we measured the AsA content again in the selected lines (data not shown) to validate the previous screening results. As expected, a high variation in AsA content was observed between the different accessions due to the heterozygous nature of spinach (dioecious outcrossed plants). The high AsA content lines were PI 648952 (H-1), PI 604783 (H-11), PI 648948 (H-2), PI 648956 (H-4), and PI 648945 (H-5), and the low AsA content lines were PI 648953 (L-11), PI 296393 (L-3), PI 169669 (L-7), PI 173972 (L-8), and PI 165994 (L-9) (Figure 3.3).

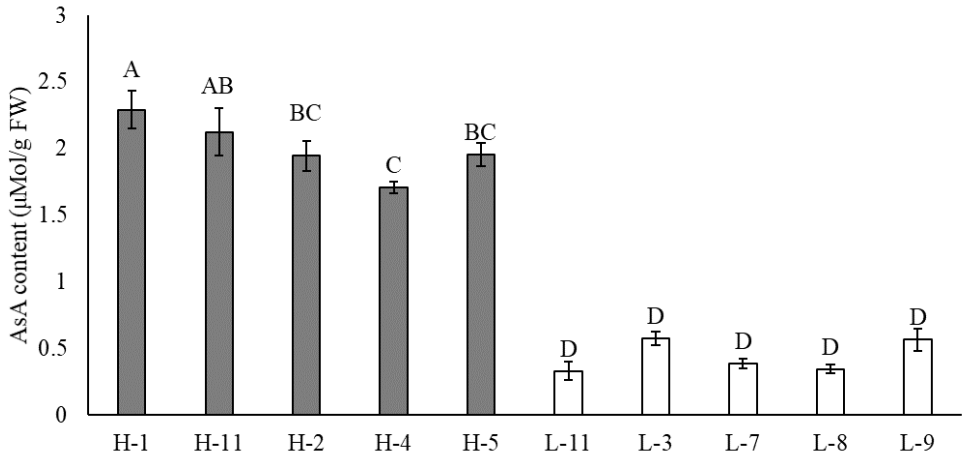

Figure 3.3 Total AsA content in the selected spinach lines for salt tolerance evaluation. H-1 = PI 648952, H-11 = PI 604783, H-2 = PI 648948, H-4 = PI 648956, H-5 = PI 648945, L-11 = PI 648953, L-3 = PI 296393, L-7 = PI 169669, L-8 = PI 173972, L-9 = PI 165994. (Mean ±SD, N = 5) Different letters indicate statistical differences between treatments at $\alpha = 0.05$.

3.4.2.2 Effect of salt stress on stomatal conductance in high- and low-AsA-content spinach lines

We evaluated the selected high- and low-AsA-content spinach lines for stomatal conductance at 1, 3, 8, 10, and 14 days of treatment with 0 and 150 mM NaCl in five-week-old plants. Plants were watered daily with 10 mL of the saline solution or plain water as the control and kept at 23°C in an 11-hr photoperiod and 120 µmol m^{-2} s^{-1} of light intensity.

We performed linear regression analyses on the salt treatment and control results at the measured time points. At 0 mM NaCl, no significant relationship between AsA content and stomatal conductance was observed at any time point measured ($p < 0.05$) (Figure 3.4).

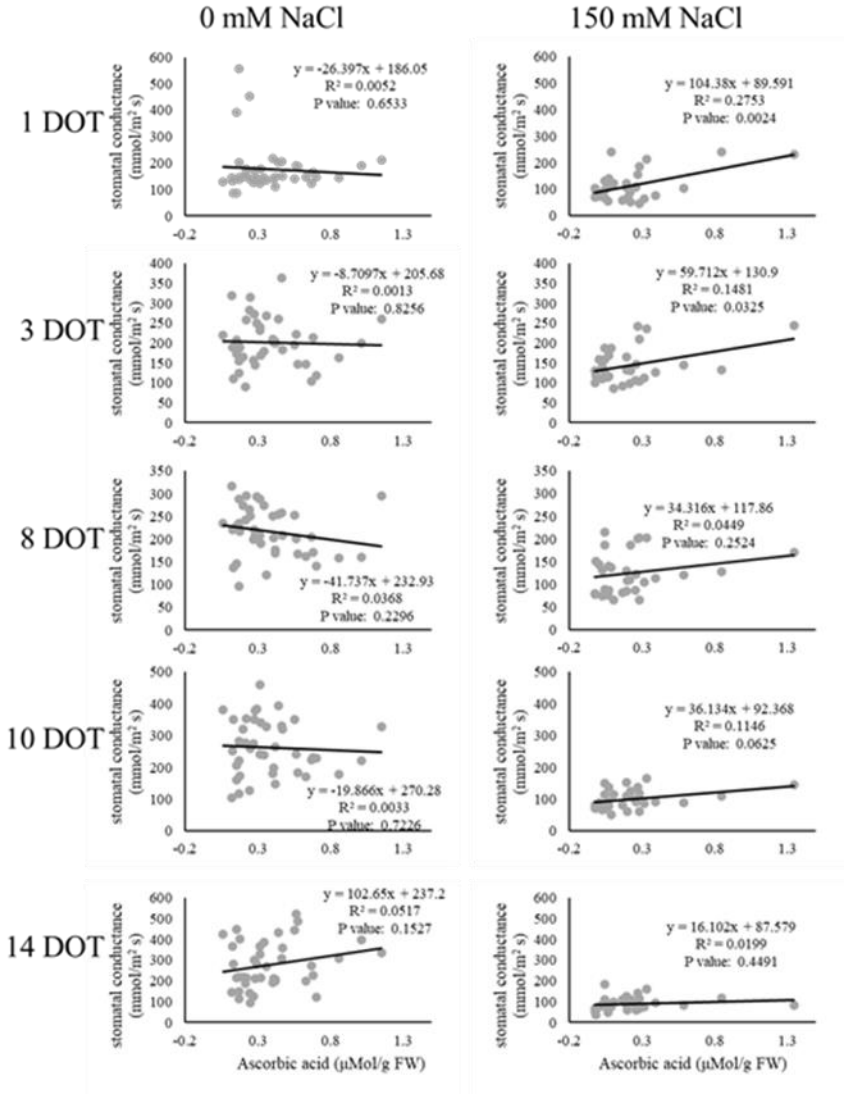

Figure 3.4 Stomatal conductance in response to salt stress in spinach. Plants were watered with either the control (0 mM NaCl) or salt solution (150 mM) for 14 days. DOT, days of treatment

On the other hand, when plants were watered with a saline solution of 150 mM NaCl, we observed a positive relationship between AsA content and stomatal conductance from 1 to 3 DOT, but no significant relationship was observed on later time points (Figure 3.4). The regression coefficient (R^2) was highest at 1 DOT ($R^2 = 0.2753$)

but decreased at subsequent time points (Figure 3.4), suggesting a potential osmotic regulation under moderate salt stress. This hypothesis agrees with previous findings on the effect of salt stress in spinach cv. Matador (Di Martino et al., 2003). The authors reported that when spinach plants are subjected to mild salt stress, nitrogen compounds, such as glycine, proline, and glycine betaine, which contribute to osmotic regulation in the cytoplasm and organelles, increase and thereby equilibrate the osmotic potential inside the salt-ion-storing vacuole. Contrarily, when the severity of the salt concentration is increased, those nitrogen compounds are no longer able to regulate the osmotic potential inside the cell (Di Martino et al., 2003). A similar study in wild soybean (*Glycine soja*) reported that in response to salt stress, glycine betaine and proline content were increased, helping to regulate the osmotic potential. Furthermore, the AsA content and Rubisco (Ribulose-1,5-bisphosphate carboxylase-oxygenase) activity also increased when salt concentrations increased (Chen et al., 2013).

When comparing between treatments, we observed a trend of a reduction in AsA content upon salt treatment. While in the no-salt control the AsA content averaged 0.3847 uMol/g FW, the AsA content averaged 0.2212 uMol/g FW with 150 mM NaCl, suggesting that the salt treatment resulted in a lower AsA content overall. In a study with strawberry (*Fragaria* × *ananassa*), plants exposed to 200 mM NaCl showed a lower stomatal conductance but a significantly higher AsA content, leading to the theory of a possible correlation between AsA content and stomatal conductance under salt stress (Crizel et al., 2020). Therefore, it is possible that spinach lines that are able to maintain a consistent AsA level upon stress can be selected for spinach salt tolerance improvement.

In this regard, a previous study from Chen and Gallie (2004) reported that transgenic tobacco with high AsA content as a result of overexpressing *Dehydroascorbate reductase* (*DHAR*) showed higher stomatal conductance as compared with non-transformed controls. Likewise, another study by Liu et al. (2011), in which the AsA synthesis gene *L-galactono-1,4-lactone dehydrogenase* (*GLDH*) was suppressed or overexpressed, reported a positive relationship between AsA content and stomatal conductance. The authors also demonstrated a positive correlation between AsA and Rubisco protein content, and the photosynthetic rate. For instance, upon overexpressing *GLDH* in transgenic rice, Rubisco content increased by 59.3% when the AsA level was 1.48 times higher than that in the wild type. Also, the net photosynthetic rate was significantly higher ($p = 0.002$) than that in the wild type (Liu et al., 2011). These findings suggest that the ability to maintain the function of the photosynthesis system depends on the amount of AsA present in the plants, whereby AsA ameliorates the negative effect of ROS produced during stress, and therefore stomata can be kept open and stomatal conductance remains high (Gallie, 2013).

3.4.2.3 Effect of salt treatment on chlorophyll fluorescence in high- and low-AsA-content spinach lines

We measured chlorophyll fluorescence as an indicator of plant stress; in this case due to the effect of the salt in the growing media on photosystem II (PSII) in the dark-adapted state (Khaleghi et al., 2012).

We performed linear regression analysis on the salt-treated and control plants having high and low AsA content. At 0 mM NaCl, no significant relationship was

observed between the AsA content and chlorophyll fluorescence at 1, 3, and 8 DOT, although a slight positive trend was observed. This positive relationship was statistically significant at days 10 and 14 ($p < 0.01$) (Figure 3.5).

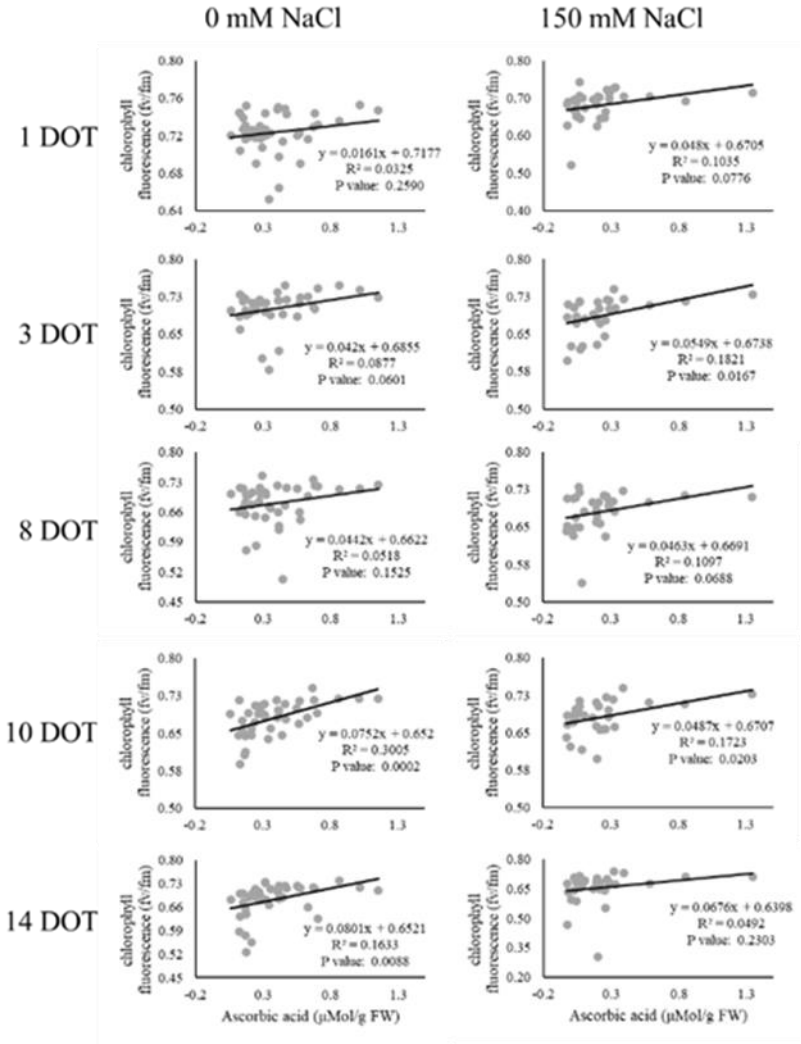

Figure 3.5 Chlorophyll fluorescence in response to salt stress in spinach. Plants were watered with either the control (0 mM NaCl) or salt solution (150 mM) for 14 days. DOT, days of treatment.

A similar relationship was observed when plants were watered with 150 mM NaCl. Although a positive relationship trend, indicating that chlorophyll fluorescence

was higher in plants with higher AsA content, was observed in all time points measured, statistical significance was only reached at days 3 and 10 at $p < 0.05$ (borderline significant at 1 and 8 DOT) (Figure 3.5). Together, the data suggest that higher AsA content in spinach results in higher energy conversion independent of salt stress. When a similar study was performed to evaluate the response of wild soybean to salt stress, the AsA content increased when the salt concentration increased, and the chlorophyll fluorescence remained unchanged between the control and the treatments. The authors proposed that this could be an indication that PSII did not suffer damage (Chen et al., 2013). Likewise, in lettuce (*Lactuca sativa*) under several NaCl concentrations, a positive and significant correlation between vitamin C and chlorophyll fluorescence was observed (Shin et al., 2020). However, if the stress is high and prolonged, PSII may shut down to reduce damage (Stepien and Johnson, 2009).

3.4.2.4 Effect of salt treatment on biomass production in the high- and low-AsA-content spinach lines

We also measured biomass indicators, such as fresh and dry weight, to assess the effect of endogenous AsA content on yield under salt stress. We measured fresh and dry weight on day 14 of the salt stress treatment and performed regression analysis to evaluate the effect of AsA content. When no salt (0 mM NaCl) was applied, the average yield was 2.26 g and 0.24 g of fresh and dry weight, respectively. The presence of salt (150 mM NaCl), however, reduced the average yield, resulting in 0.83 g and 0.11 g for the fresh and dry weight, respectively. At 14 DOT, no significant relationship ($p = 0.05$) was observed when plants were treated with 0 or 150 mM NaCl. However, a trend was

observed, suggesting that under salt stress, high AsA content resulted in higher biomass as measured by fresh and dry weight. We can speculate that with higher AsA content and higher salt concentration (e.g. 350 mM NaCl as observed in Figure 3.2A) , the effect on biomass production will be larger, potentially resulting in a significant positive relationship (Figure 3.6).

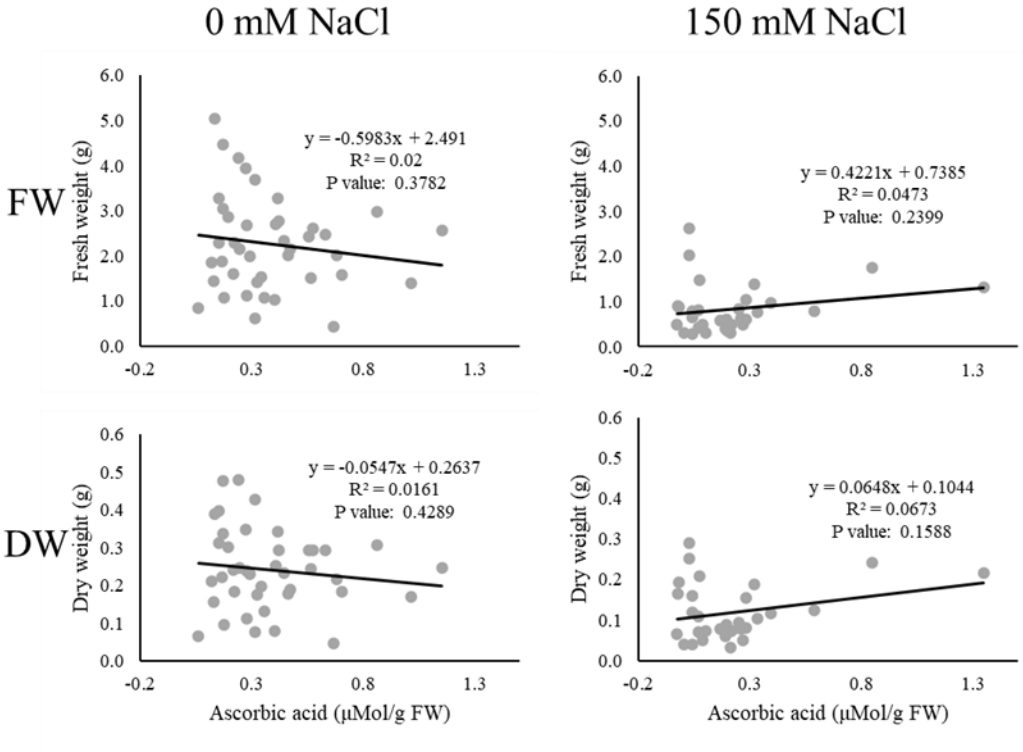

Figure 3.6 Effect on yield in high- and low-AsA-content spinach accessions at 14 days of salt treatment. FW, fresh weight. DW, dry weight.

This hypothesis is supported by previous studies in which overexpression of the AsA synthesis gene *L-Galactono-1, 4-lactone dehydrogenase* (*GalLDH*) in transgenic tobacco cv. Xanthin plants resulted in increased AsA content and significantly higher fresh weight than wild-type plants after treatment with 100 mM NaCl (Liu et al., 2013).

Likewise, overexpression of the AsA recycling gene *Monodehydroascorbate reductase* (*MDHAR*) in tomato cv. Zhongshu 4 resulted in higher fresh weight and chlorophyll fluorescence under salt stress along with a higher AsA content when compared to the wild type and knocked-down transgenic plants (Li et al., 2012a). While enhancement of AsA results in higher biomass, the Arabidopsis vitamin C-deficient mutant *vtc* accumulated 30% less AsA in leaf tissue, leading to reduced fresh and dry weight even when no oxidative stress was present, further corroborating the effect of AsA on plant biomass (Veljovic-Jovanovic et al., 2001).

3.5 Conclusions

Saline soils are detrimental for spinach cultivation, resulting in a dramatic reduction in biomass production. This reduction is exacerbated as the salt concentration increases. However, AsA can ameliorate the negative effects of salt stress by maintaining stomatal conductance and chlorophyll fluorescence, although it was not reflected in a significant effect on biomass production at 150 mM NaCl. While several studies have shown that plants overexpressing AsA biosynthesis or recycling genes can withstand higher levels of abiotic stress, our study suggests that salt stress tolerance can also be obtained using natural variation in spinach germplasm. However, further work is needed to determine the effect of AsA content on salt stress at higher salt concentrations using genotypes with uniform low and high AsA content. Recurrent mass selection can be used as an approach to reduce plant to plant variation within spinach lines in future experiments.

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APPENDIX A

A.1 Supplementary Material

Supplemental table 1. Ascorbic acid content in Spinach GWAS germplasm panel

USDA Accession ID	Line ID	AsA content	AsA Avg
CPPSIH 3 01	WR-1	0.14569	
CPPSIH 3 01	WR-1	0.12871	0.14141
CPPSIH 3 01	WR-1	0.14985	
CPPSIH 3 02	WR-2	0.11591	
CPPSIH 3 02	WR-2	0.13278	0.11064
CPPSIH 3 02	WR-2	0.08325	
CPPSIH 3 03	WR-3	0.20860	
CPPSIH 3 03	WR-3	0.08741	0.12585
CPPSIH 3 03	WR-3	0.08152	
CPPSIH 3 04	WR-4	0.11279	
CPPSIH 3 04	WR-4	0.21345	0.15813
CPPSIH 3 04	WR-4	0.14816	
CPPSIH 3 05	WR-5	0.30453	
CPPSIH 3 05	WR-5	0.16493	0.20672
CPPSIH 3 05	WR-5	0.15071	
CPPSIH 3 06	WR-6	0.40344	
CPPSIH 3 06	WR-6	0.11853	0.22602
CPPSIH 3 06	WR-6	0.15609	
CPPSIH 3 07	WR-7	0.14985	
CPPSIH 3 07	WR-7	0.07387	0.11431
CPPSIH 3 07	WR-7	0.11920	
CPPSIH 3 08	WR-8	0.22680	
CPPSIH 3 08	WR-8	0.13448	0.15241
CPPSIH 3 08	WR-8	0.09594	
CPPSIH 3 09	WR-9	0.03520	
CPPSIH 3 09	WR-9	0.32780	0.14285
CPPSIH 3 09	WR-9	0.06556	
NSL 6082	WR-10	0.06901	
NSL 6082	WR-10	0.08940	
NSL 6082	WR-10	0.25276	0.13505
NSL 6082	WR-10	0.10870	
NSL 6082	WR-10	0.15540	

NSL 6083	WR-11	0.63235	
NSL 6083	WR-11	0.07136	0.25819
NSL 6083	WR-11	0.07088	
NSL 6085	WR-12	0.07657	0.12127
NSL 6085	WR-12	0.16597	
NSL 6087	WR-13	0.20434	
NSL 6087	WR-13	0.09043	0.12707
NSL 6087	WR-13	0.08645	
NSL 6088	WR-14	0.08693	
NSL 6088	WR-14	0.02790	0.09655
NSL 6088	WR-14	0.17483	
NSL 6089	WR-15	0.15517	0.10808
NSL 6089	WR-15	0.06099	
NSL 6090	WR-16	0.49170	
NSL 6090	WR-16	0.29502	0.44276
NSL 6090	WR-16	0.54158	
NSL 6092	WR-17	0.11042	
NSL 6092	WR-17	0.25854	0.21570
NSL 6092	WR-17	0.27813	
NSL 6093	WR-18	0.03032	
NSL 6093	WR-18	0.03341	0.04218
NSL 6093	WR-18	0.06281	
NSL 6094	WR-19	0.06556	
NSL 6094	WR-19	0.12871	0.09066
NSL 6094	WR-19	0.07770	
NSL 6095	WR-20	0.16188	
NSL 6095	WR-20	0.32780	0.20834
NSL 6095	WR-20	0.13535	
NSL 6096	WR-21	0.11003	
NSL 6096	WR-21	0.36657	0.22431
NSL 6096	WR-21	0.19633	
NSL 6099	WR-22	0.13112	
NSL 6099	WR-22	0.15158	0.17193
NSL 6099	WR-22	0.23310	
NSL 6557	WR-23	0.13535	
NSL 6557	WR-23	0.14985	0.11563
NSL 6557	WR-23	0.06170	
NSL 6693	WR-24	0.03773	0.22168
NSL 6693	WR-24	0.42814	

NSL 6693	WR-24	0.19917	
NSL 6782	WR-25	0.11080	
NSL 6782	WR-25	0.12057	0.12344
NSL 6782	WR-25	0.13893	
NSL 22003	WR-26	0.74006	
NSL 22003	WR-26	0.18190	0.41721
NSL 22003	WR-26	0.32967	
NSL 22149	WR-27	0.06435	
NSL 22149	WR-27	0.42421	0.26630
NSL 22149	WR-27	0.31034	
NSL 28216	WR-28	0.38981	
NSL 28216	WR-28	0.24975	0.27225
NSL 28216	WR-28	0.17719	
NSL 28217	WR-29	0.20703	
NSL 28217	WR-29	0.11485	0.14892
NSL 28217	WR-29	0.12488	
NSL 28218	WR-30	0.10560	
NSL 28218	WR-30	0.09478	0.12075
NSL 28218	WR-30	0.16188	
NSL 31384	WR-31	0.11786	
NSL 31384	WR-31	0.09896	0.08157
NSL 31384	WR-31	0.02790	
NSL 32678	WR-32	0.43428	
NSL 32678	WR-32	0.14985	0.22353
NSL 32678	WR-32	0.08645	
NSL 37031	WR-33	0.03473	
NSL 37031	WR-33	0.16810	0.23188
NSL 37031	WR-33	0.49280	
NSL 40592	WR-34	0.26412	
NSL 40592	WR-34	0.52100	0.28329
NSL 40592	WR-34	0.06475	
NSL 65915	WR-35	0.26820	
NSL 65915	WR-35	0.09201	0.17933
NSL 65915	WR-35	0.17779	
NSL 68263	WR-36	0.05550	
NSL 68263	WR-36	0.14732	0.07743
NSL 68263	WR-36	0.02946	
NSL 68264	WR-37	0.12792	0.14990
NSL 68264	WR-37	0.25276	

NSL 68264	WR-37	0.06901	
NSL 81328	WR-38	0.45047	
NSL 81328	WR-38	0.30065	0.28295
NSL 81328	WR-38	0.09773	
NSL 81329	WR-39	0.39641	
NSL 81329	WR-39	0.08991	0.17450
NSL 81329	WR-39	0.03720	
NSL 92009	WR-40	0.61253	
NSL 92009	WR-40	0.20840	0.30562
NSL 92009	WR-40	0.09594	
NSL 184379	WR-41	0.03450	
NSL 184379	WR-41	0.29300	0.13799
NSL 184379	WR-41	0.08645	
NSL 184380	WR-42	0.21984	
NSL 184380	WR-42	0.74925	0.40198
NSL 184380	WR-42	0.23686	
NSL 184398	WR-43	0.17483	
NSL 184398	WR-43	0.06597	0.10373
NSL 184398	WR-43	0.07040	
NSL 186328	WR-44	0.11320	
NSL 186328	WR-44	0.41251	0.20521
NSL 186328	WR-44	0.08991	
PI 103063	WR-45	0.32051	
PI 103063	WR-45	0.53596	0.39861
PI 103063	WR-45	0.33937	
PI 163309	WR-46	0.05926	
PI 163309	WR-46	0.22928	0.16053
PI 163309	WR-46	0.19306	
PI 163310	WR-47	0.03032	
PI 163310	WR-47	0.16015	0.14177
PI 163310	WR-47	0.23484	
PI 164965	WR-48	0.11219	
PI 164965	WR-48	0.07335	0.08304
PI 164965	WR-48	0.06357	
PI 164966	WR-49	0.07335	0.16071
PI 164966	WR-49	0.24806	
PI 165012	WR-50	0.06767	0.05023
PI 165012	WR-50	0.03278	
PI 165043	WR-51	0.06475	0.13548

PI 165043	WR-51	0.17140	
PI 165043	WR-51	0.17028	
PI 165560	WR-53	0.24537	
PI 165560	WR-53	0.06767	0.18804
PI 165560	WR-53	0.25108	
PI 165710	WR-54	0.02997	
PI 165710	WR-54	0.26370	0.20593
PI 165710	WR-54	0.32411	
PI 165994	WR-55	0.15426	
PI 165994	WR-55	0.11080	0.13665
PI 165994	WR-55	0.14488	
PI 166044	WR-56	0.11988	
PI 166044	WR-56	0.17483	0.13343
PI 166044	WR-56	0.10560	
PI 166366	WR-57	0.18296	
PI 166366	WR-57	0.06244	0.33853
PI 166366	WR-57	0.77021	
PI 167098	WR-58	0.27766	
PI 167098	WR-58	0.03218	0.17985
PI 167098	WR-58	0.22970	
PI 167194	WR-59	0.06063	
PI 167194	WR-59	0.02850	0.06564
PI 167194	WR-59	0.10777	
PI 167195	WR-60	0.18866	
PI 167195	WR-60	0.12792	0.16459
PI 167195	WR-60	0.17719	
PI 167434	WR-61	0.09896	
PI 167434	WR-61	0.60186	0.30172
PI 167434	WR-61	0.20434	
PI 167434	WR-61	0.28608	
PI 167434	WR-61	0.05701	0.16098
PI 167434	WR-61	0.13986	
PI 169026	WR-62	0.22523	
PI 169026	WR-62	0.13712	0.23081
PI 169026	WR-62	0.33009	
PI 169668	WR-63	0.11159	
PI 169668	WR-63	0.12792	0.15024
PI 169668	WR-63	0.21120	
PI 169669	WR-64	0.11159	0.10597

PI 169669	WR-64	0.06063	
PI 169669	WR-64	0.14569	
PI 169670	WR-65	0.14468	
PI 169670	WR-65	0.18190	0.21170
PI 169670	WR-65	0.30852	
PI 169671	WR-66	0.27070	
PI 169671	WR-66	0.18731	0.20206
PI 169671	WR-66	0.14816	
PI 169673	WR-67	0.12871	
PI 169673	WR-67	0.06099	0.11146
PI 169673	WR-67	0.14468	
PI 169674	WR-68	0.06396	
PI 169674	WR-68	0.19306	
PI 169674	WR-68	0.07040	0.13218
PI 169674	WR-68	0.13362	
PI 169674	WR-68	0.13535	
PI 169674	WR-68	0.19668	
PI 169675	WR-69	0.07601	
PI 169675	WR-69	0.31526	0.19242
PI 169675	WR-69	0.18598	
PI 169676	WR-70	0.26370	
PI 169676	WR-70	0.25854	0.20686
PI 169676	WR-70	0.09834	
PI 169677	WR-71	0.06435	
PI 169677	WR-71	0.03885	0.08191
PI 169677	WR-71	0.14252	
PI 169678	WR-72	0.26038	
PI 169678	WR-72	0.10022	0.15662
PI 169678	WR-72	0.10927	
PI 169679	WR-73	0.11485	
PI 169679	WR-73	0.28265	
PI 169679	WR-73	0.26820	0.18560
PI 169679	WR-73	0.28350	
PI 169679	WR-73	0.03568	
PI 169679	WR-73	0.12871	
PI 169680	WR-74	0.06767	
PI 169680	WR-74	0.06319	0.05435
PI 169680	WR-74	0.03218	
PI 169682	WR-75	0.09310	0.11130

PI 169682	WR-75	0.05994	
PI 169682	WR-75	0.18085	
PI 169683	WR-76	0.58683	0.58683
PI 169685	WR-78	0.66434	0.88619
PI 169685	WR-78	1.10805	
PI 169688	WR-79	0.91692	
PI 169688	WR-79	0.96992	0.95970
PI 169688	WR-79	0.99225	
PI 169689	WR-80	0.60244	0.60244
PI 169690	WR-81	1.07769	
PI 169690	WR-81	1.00584	1.01017
PI 169690	WR-81	0.94697	
PI 171858	WR-82	0.87413	0.95440
PI 171858	WR-82	1.03468	
PI 171859	WR-83	0.89197	
PI 171859	WR-83	1.00431	1.09909
PI 171859	WR-83	1.40100	
PI 171860	WR-84	0.84950	0.77076
PI 171860	WR-84	0.69202	
PI 171861	WR-85	0.95039	
PI 171861	WR-85	1.10805	0.89544
PI 171861	WR-85	0.62789	
PI 171862	WR-86	0.97661	
PI 171862	WR-86	0.84356	1.00037
PI 171862	WR-86	1.18094	
PI 171864	WR-87	0.99225	
PI 171864	WR-87	1.10805	1.13101
PI 171864	WR-87	1.29272	
PI 171865	WR-88	0.96332	
PI 171865	WR-88	1.75998	1.42622
PI 171865	WR-88	1.55534	
PI 171866	WR-89	0.53156	0.97935
PI 171866	WR-89	1.42714	
PI 171867	WR-90	1.26719	0.93481
PI 171867	WR-90	0.60244	
PI 173122	WR-91	0.11159	0.76112
PI 173122	WR-91	1.41066	
PI 173123	WR-92	0.20703	0.15159
PI 173123	WR-92	0.06811	

PI 173123	WR-92	0.17961	
PI 173124	WR-93	0.93102	0.93102
PI 173125	WR-94	1.28368	1.28368
PI 173126	WR-95	0.37914	0.76172
PI 173126	WR-95	1.14431	
PI 173127	WR-96	0.35760	0.80632
PI 173127	WR-96	1.13581	
PI 173127	WR-96	0.92555	
PI 173128	WR-97	0.07770	0.09510
PI 173128	WR-97	0.06681	
PI 173128	WR-97	0.14080	
PI 173129	WR-98	1.31987	1.54730
PI 173129	WR-98	1.06899	
PI 173129	WR-98	2.25303	
PI 173130	WR-99	0.93888	0.97942
PI 173130	WR-99	0.98725	
PI 173130	WR-99	1.01215	
PI 173131	WR-100	1.21885	1.21885
PI 173809	WR-101	1.04895	1.31985
PI 173809	WR-101	1.45371	
PI 173809	WR-101	1.45688	
PI 173972	WR-102	0.11655	0.09495
PI 173972	WR-102	0.07335	
PI 174383	WR-103	0.06170	0.48129
PI 174383	WR-103	0.98559	
PI 174383	WR-103	0.14671	
PI 174383	WR-103	0.11003	
PI 174383	WR-103	1.10240	
PI 174384	WR-104	0.17367	0.17956
PI 174384	WR-104	0.15990	
PI 174384	WR-104	0.20510	
PI 174385	WR-105	0.77021	0.92524
PI 174385	WR-105	1.07809	
PI 174385	WR-105	0.92743	
PI 174386	WR-106	0.10022	0.26486
PI 174386	WR-106	0.13712	
PI 174386	WR-106	0.55726	
PI 174387	WR-107	0.19546	0.16053
PI 174387	WR-107	0.13278	

PI 174387	WR-107	0.15336	
PI 174389	WR-108	0.14409	
PI 174389	WR-108	0.22946	0.19779
PI 174389	WR-108	0.21984	
PI 174960	WR-109	0.30493	
PI 174960	WR-109	0.09896	0.22913
PI 174960	WR-109	0.28350	
PI 175311	WR-110	0.09834	
PI 175311	WR-110	0.30404	
PI 175311	WR-110	0.12562	0.17744
PI 175311	WR-110	0.30852	
PI 175311	WR-110	0.11159	
PI 175311	WR-110	0.11655	
PI 175312	WR-111	0.08940	
PI 175312	WR-111	0.24975	0.12271
PI 175312	WR-111	0.02898	
PI 175313	WR-112	0.14080	
PI 175313	WR-112	0.23840	
PI 175313	WR-112	0.06063	0.17500
PI 175313	WR-112	0.20172	
PI 175313	WR-112	0.18731	
PI 175313	WR-112	0.22116	
PI 175595	WR-113	0.22558	
PI 175595	WR-113	0.25654	0.22506
PI 175595	WR-113	0.19306	
PI 175923	WR-114	0.11988	
PI 175923	WR-114	0.16739	
PI 175923	WR-114	0.02835	0.18712
PI 175923	WR-114	0.16390	
PI 175923	WR-114	0.45607	
PI 175924	WR-115	0.23384	0.16671
PI 175924	WR-115	0.09958	
PI 175925	WR-116	0.51366	
PI 175925	WR-116	0.35438	0.31624
PI 175925	WR-116	0.08069	
PI 175926	WR-117	0.13712	
PI 175926	WR-117	0.43428	0.25269
PI 175926	WR-117	0.14468	
PI 175926	WR-117	0.32576	

PI 175926	WR-117	0.22161	
PI 175927	WR-118	0.05893	0.08774
PI 175927	WR-118	0.11655	
PI 175928	WR-119	0.02835	
PI 175928	WR-119	0.16493	0.10630
PI 175928	WR-119	0.12562	
PI 175930	WR-120	0.17982	
PI 175930	WR-120	0.12127	0.14199
PI 175930	WR-120	0.12488	
PI 175931	WR-121	0.47680	
PI 175931	WR-121	0.14879	0.27534
PI 175931	WR-121	0.20044	
PI 175932	WR-122	0.15990	
PI 175932	WR-122	0.14985	0.22420
PI 175932	WR-122	0.36284	
PI 176371	WR-123	0.34020	
PI 176371	WR-123	0.25674	0.23987
PI 176371	WR-123	0.12268	
PI 176372	WR-124	0.03568	0.07363
PI 176372	WR-124	0.11159	
PI 176769	WR-125	0.09896	
PI 176769	WR-125	0.12197	0.12476
PI 176769	WR-125	0.15336	
PI 176770	WR-126	0.13194	
PI 176770	WR-126	0.11239	0.13083
PI 176770	WR-126	0.14816	
PI 176771	WR-127	0.13802	0.11556
PI 176771	WR-127	0.09310	
PI 176772	WR-128	0.18085	
PI 176772	WR-128	0.11402	0.18924
PI 176772	WR-128	0.27285	
PI 176773	WR-129	0.36805	
PI 176773	WR-129	0.31894	0.31314
PI 176773	WR-129	0.25242	
PI 176774	WR-130	0.49170	
PI 176774	WR-130	0.24476	0.29944
PI 176774	WR-130	0.16188	
PI 176775	WR-131	0.14879	0.12775
PI 176775	WR-131	0.11527	

PI 176775	WR-131	0.11920	
PI 176777	WR-132	0.33738	
PI 176777	WR-132	0.06556	0.19259
PI 176777	WR-132	0.17483	
PI 176778	WR-133	0.06281	
PI 176778	WR-133	0.18296	0.12405
PI 176778	WR-133	0.12638	
PI 176780	WR-135	0.09255	0.09255
PI 177081	WR-136	0.41135	0.60151
PI 177081	WR-136	0.79166	
PI 177082	WR-137	0.29875	
PI 177082	WR-137	0.10851	0.28144
PI 177082	WR-137	0.43706	
PI 177557	WR-138	0.14732	
PI 177557	WR-138	0.40344	0.25040
PI 177557	WR-138	0.20044	
PI 177558	WR-139	0.02790	
PI 177558	WR-139	0.18866	0.09647
PI 177558	WR-139	0.07284	
PI 179041	WR-140	0.08598	
PI 179041	WR-140	0.23996	0.17146
PI 179041	WR-140	0.18843	
PI 179044	WR-141	0.16703	
PI 179044	WR-141	0.20396	0.33296
PI 179044	WR-141	0.62789	
PI 179507	WR-142	0.26224	
PI 179507	WR-142	0.06724	0.23470
PI 179507	WR-142	0.37463	
PI 179508	WR-143	0.03014	
PI 179508	WR-143	0.11786	0.10537
PI 179508	WR-143	0.16810	
PI 179509	WR-144	0.10086	
PI 179509	WR-144	0.06856	0.11891
PI 179509	WR-144	0.18731	
PI 179588	WR-145	0.22250	
PI 179588	WR-145	0.06357	0.14161
PI 179588	WR-145	0.13875	
PI 179589	WR-146	0.12715	0.18303
PI 179589	WR-146	0.29631	

PI 179589	WR-146	0.12562	
PI 179590	WR-147	0.09536	
PI 179590	WR-147	0.10284	0.11306
PI 179590	WR-147	0.14099	
PI 179591	WR-148	0.15797	
PI 179591	WR-148	0.03450	0.12486
PI 179591	WR-148	0.18211	
PI 179592	WR-149	0.18511	
PI 179592	WR-149	0.23572	0.25612
PI 179592	WR-149	0.34754	
PI 179593	WR-150	0.11464	
PI 179593	WR-150	0.17679	0.18647
PI 179593	WR-150	0.26798	
PI 179594	WR-151	0.06134	
PI 179594	WR-151	0.34965	0.24443
PI 179594	WR-151	0.32230	
PI 179595	WR-152	0.34137	
PI 179595	WR-152	0.09773	0.18901
PI 179595	WR-152	0.12792	
PI 179596	WR-153	0.07136	
PI 179596	WR-153	0.19570	0.09974
PI 179596	WR-153	0.03218	
PI 179597	WR-154	0.30186	
PI 179597	WR-154	0.05609	0.14503
PI 179597	WR-154	0.07713	
PI 181086	WR-155	0.09653	
PI 181086	WR-155	0.10351	0.10758
PI 181086	WR-155	0.12268	
PI 181808	WR-156	0.06901	
PI 181808	WR-156	0.08889	0.13897
PI 181808	WR-156	0.25900	
PI 181809	WR-157	0.30258	
PI 181809	WR-157	0.17010	0.42481
PI 181809	WR-157	0.80175	
PI 181923	WR-158	0.37022	
PI 181923	WR-158	0.13802	0.29972
PI 181923	WR-158	0.39091	
PI 181964	WR-159	0.03746	0.12911
PI 181964	WR-159	0.20172	

PI 181964	WR-159	0.14816	
PI 183246	WR-160	0.11527	
PI 183246	WR-160	0.11720	0.17707
PI 183246	WR-160	0.29875	
PI 184137	WR-161	0.13278	
PI 184137	WR-161	0.12127	0.09503
PI 184137	WR-161	0.03103	
PI 192945	WR-162	0.29757	
PI 192945	WR-162	0.17839	0.24484
PI 192945	WR-162	0.25854	
PI 193618	WR-163	0.08645	
PI 193618	WR-163	0.09773	0.16931
PI 193618	WR-163	0.32375	
PI 193619	WR-164	0.60371	0.35229
PI 193619	WR-164	0.10086	
PI 200882	WR-165	0.06993	
PI 200882	WR-165	0.06947	0.09758
PI 200882	WR-165	0.15336	
PI 204632	WR-166	0.22639	
PI 204632	WR-166	0.28097	
PI 204632	WR-166	0.14175	0.23546
PI 204632	WR-166	0.38720	
PI 204632	WR-166	0.14099	
PI 204732	WR-167	0.07828	
PI 204732	WR-167	0.25584	0.17272
PI 204732	WR-167	0.18403	
PI 204733	WR-168	0.35964	
PI 204733	WR-168	0.20302	0.22096
PI 204733	WR-168	0.10022	
PI 204734	WR-169	0.10927	
PI 204734	WR-169	0.77327	0.36702
PI 204734	WR-169	0.21853	
PI 204735	WR-170	0.33959	
PI 204735	WR-170	0.16919	0.21954
PI 204735	WR-170	0.14985	
PI 204736	WR-171	0.33348	
PI 204736	WR-171	0.21596	0.24750
PI 204736	WR-171	0.19306	
PI 205231	WR-172	0.11402	0.51440

PI 205231	WR-172	0.91478	
PI 205232	WR-173	0.34137	
PI 205232	WR-173	0.25320	0.26334
PI 205232	WR-173	0.19546	
PI 205233	WR-174	0.03103	
PI 205233	WR-174	0.30453	0.18321
PI 205233	WR-174	0.21407	
PI 205234	WR-175	0.52758	
PI 205234	WR-175	0.11988	0.26978
PI 205234	WR-175	0.16188	
PI 205235	WR-176	0.10927	
PI 205235	WR-176	0.41574	
PI 205235	WR-176	0.13712	0.20410
PI 205235	WR-176	0.13986	
PI 205235	WR-176	0.21853	
PI 206007	WR-177	0.38612	
PI 206007	WR-177	0.15336	0.22221
PI 206007	WR-177	0.12715	
PI 206474	WR-178	0.17140	
PI 206474	WR-178	0.24253	0.23276
PI 206474	WR-178	0.28435	
PI 206475	WR-179	0.11485	0.11485
PI 206753	WR-180	0.39140	
PI 206753	WR-180	0.23996	0.25471
PI 206753	WR-180	0.13278	
PI 207518	WR-181	0.07770	
PI 207518	WR-181	0.11080	
PI 207518	WR-181	0.03428	0.31847
PI 207518	WR-181	1.06725	
PI 207518	WR-181	0.07040	
PI 207518	WR-181	0.55038	
PI 209644	WR-182	0.11786	
PI 209644	WR-182	0.13623	
PI 209644	WR-182	0.22523	0.27468
PI 209644	WR-182	0.57873	
PI 209644	WR-182	0.14369	
PI 209644	WR-182	0.44636	
PI 209645	WR-183	0.03406	0.11393
PI 209645	WR-183	0.13362	

PI 209645	WR-183	0.07088	
PI 209645	WR-183	0.23572	
PI 209645	WR-183	0.09536	
PI 209646	WR-184	0.10217	
PI 209646	WR-184	1.22980	
PI 209646	WR-184	0.03122	0.72822
PI 209646	WR-184	1.06266	
PI 209646	WR-184	1.21525	
PI 209647	WR-185	0.03544	
PI 209647	WR-185	0.45475	0.20938
PI 209647	WR-185	0.25741	
PI 209647	WR-185	0.08991	
PI 211632	WR-186	1.16219	
PI 211632	WR-186	0.25674	0.66723
PI 211632	WR-186	0.58275	
PI 212119	WR-187	0.10927	
PI 212119	WR-187	0.51242	0.30109
PI 212119	WR-187	0.28160	
PI 212120	WR-188	0.05492	
PI 212120	WR-188	0.06681	
PI 212120	WR-188	0.08195	0.19379
PI 212120	WR-188	0.24253	
PI 212120	WR-188	0.03668	
PI 212120	WR-188	0.67988	
PI 212328	WR-189	0.03668	
PI 212328	WR-189	0.70958	0.31010
PI 212328	WR-189	0.18403	
PI 212921	WR-190	0.06681	0.10676
PI 212921	WR-190	0.14671	
PI 217425	WR-191	0.23840	
PI 217425	WR-191	0.45939	
PI 217425	WR-191	0.23440	0.40359
PI 217425	WR-191	0.29465	
PI 217425	WR-191	0.37732	
PI 217425	WR-191	0.81736	
PI 219949	WR-192	0.61531	
PI 219949	WR-192	0.97944	0.54103
PI 219949	WR-192	0.02835	
PI 220121	WR-193	0.26079	0.12607

PI 220121	WR-193	0.03238	
PI 220121	WR-193	0.08505	
PI 220546	WR-194	0.47068	
PI 220546	WR-194	0.33156	0.27808
PI 220546	WR-194	0.03198	
PI 220686	WR-195	0.10420	
PI 220686	WR-195	0.36171	0.20635
PI 220686	WR-195	0.15313	
PI 222270	WR-196	0.66313	
PI 222270	WR-196	0.14271	0.29147
PI 222270	WR-196	0.06856	
PI 222749	WR-197	0.39246	
PI 222749	WR-197	0.63878	0.52721
PI 222749	WR-197	0.55038	
PI 222750	WR-198	0.11853	
PI 222750	WR-198	0.07284	0.12623
PI 222750	WR-198	0.18731	
PI 222838	WR-199	0.59599	
PI 222838	WR-199	0.09201	0.25379
PI 222838	WR-199	0.07335	
PI 223536	WR-200	0.03473	0.19879
PI 223536	WR-200	0.36284	
PI 224959	WR-201	0.55944	
PI 224959	WR-201	0.23534	0.39070
PI 224959	WR-201	0.37732	
PI 226671	WR-202	0.86810	
PI 226671	WR-202	0.12638	0.41648
PI 226671	WR-202	0.25495	
PI 227045	WR-203	0.17252	
PI 227045	WR-203	0.10560	0.11768
PI 227045	WR-203	0.07493	
PI 227230	WR-204	0.23090	
PI 227230	WR-204	0.10851	0.13776
PI 227230	WR-204	0.07387	
PI 227383	WR-205	0.23534	
PI 227383	WR-205	0.40917	0.26942
PI 227383	WR-205	0.24806	
PI 227383	WR-205	0.18511	
PI 229731	WR-206	0.20284	0.13005

PI 229731	WR-206	0.03746	
PI 229731	WR-206	0.14985	
PI 229792	WR-207	0.15990	
PI 229792	WR-207	0.10351	0.13206
PI 229792	WR-207	0.13278	
PI 249920	WR-208	0.03384	
PI 249920	WR-208	0.03450	0.07189
PI 249920	WR-208	0.14732	
PI 251507	WR-209	0.03299	
PI 251507	WR-209	0.03801	
PI 251507	WR-209	0.14080	0.17697
PI 251507	WR-209	0.14468	
PI 251507	WR-209	0.18085	
PI 251507	WR-209	0.52448	
PI 256079	WR-210	0.69202	0.37704
PI 256079	WR-210	0.06207	
PI 256080	WR-211	0.39788	
PI 256080	WR-211	0.20840	0.28247
PI 256080	WR-211	0.24114	
PI 261787	WR-212	0.18085	
PI 261787	WR-212	0.17982	0.19137
PI 261787	WR-212	0.21345	
PI 261788	WR-213	1.30162	
PI 261788	WR-213	0.55533	0.71720
PI 261788	WR-213	0.29465	
PI 261789	WR-214	1.48551	0.75904
PI 261789	WR-214	0.03258	
PI 262161	WR-215	0.14569	
PI 262161	WR-215	0.15797	0.22962
PI 262161	WR-215	0.38521	
PI 262911	WR-216	0.46620	0.38940
PI 262911	WR-216	0.31260	
PI 263873	WR-217	0.09896	
PI 263873	WR-217	0.08741	0.13348
PI 263873	WR-217	0.21407	
PI 266926	WR-218	0.19917	
PI 266926	WR-218	0.15426	0.68372
PI 266926	WR-218	1.69772	
PI 274042	WR-219	0.47022	0.54073

PI 274042	WR-219	0.03746	
PI 274042	WR-219	1.11451	
PI 274044	WR-220	0.32576	
PI 274044	WR-220	0.06063	0.40262
PI 274044	WR-220	0.82147	
PI 274046	WR-221	1.18007	
PI 274046	WR-221	0.13802	0.60039
PI 274046	WR-221	0.48307	
PI 274047	WR-222	0.09422	
PI 274047	WR-222	0.10851	0.17616
PI 274047	WR-222	0.32576	
PI 274048	WR-223	0.07284	
PI 274048	WR-223	0.45475	0.29399
PI 274048	WR-223	0.35438	
PI 274049	WR-224	1.32626	
PI 274049	WR-224	0.11920	1.06079
PI 274049	WR-224	1.73690	
PI 274050	WR-225	0.18190	
PI 274050	WR-225	0.15071	0.28366
PI 274050	WR-225	0.51838	
PI 274051	WR-226	0.15426	
PI 274051	WR-226	0.17982	0.32860
PI 274051	WR-226	0.65172	
PI 274052	WR-227	0.20510	
PI 274052	WR-227	0.40868	0.22672
PI 274052	WR-227	0.06639	
PI 274053	WR-228	0.39185	
PI 274053	WR-228	0.56431	0.36034
PI 274053	WR-228	0.12488	
PI 274055	WR-229	0.14099	
PI 274055	WR-229	0.14774	
PI 274055	WR-229	0.11402	0.20159
PI 274055	WR-229	0.49280	
PI 274055	WR-229	0.11239	
PI 274056	WR-230	0.06357	
PI 274056	WR-230	0.47991	
PI 274056	WR-230	0.03238	0.12925
PI 274056	WR-230	0.03319	
PI 274056	WR-230	0.03720	

PI 274057	WR-231	0.08991	
PI 274057	WR-231	0.11786	0.16127
PI 274057	WR-231	0.27604	
PI 274058	WR-232	0.06134	
PI 274058	WR-232	0.08281	0.07858
PI 274058	WR-232	0.03032	
PI 274058	WR-232	0.13986	
PI 274059	WR-233	0.09594	
PI 274059	WR-233	0.28738	
PI 274059	WR-233	0.06357	0.13691
PI 274059	WR-233	0.10490	
PI 274059	WR-233	0.13278	
PI 274061	WR-234	0.35394	
PI 274061	WR-234	0.94471	0.59646
PI 274061	WR-234	0.49074	
PI 274063	WR-235	0.02790	
PI 274063	WR-235	0.25495	0.10514
PI 274063	WR-235	0.03258	
PI 274065	WR-236	0.49280	
PI 274065	WR-236	1.09266	0.60900
PI 274065	WR-236	0.24153	
PI 274311	WR-237	0.09366	
PI 274311	WR-237	0.15426	0.11318
PI 274311	WR-237	0.11003	
PI 274311	WR-237	0.09478	
PI 286435	WR-238	0.02850	
PI 286435	WR-238	0.15797	0.11063
PI 286435	WR-238	0.08238	
PI 286435	WR-238	0.17367	
PI 303138	WR-240	0.15990	
PI 303138	WR-240	0.23090	0.16500
PI 303138	WR-240	0.10420	
PI 319220	WR-241	0.58618	
PI 319220	WR-241	0.22523	0.29303
PI 319220	WR-241	0.06767	
PI 321020	WR-242	0.61513	
PI 321020	WR-242	1.00431	0.63216
PI 321020	WR-242	0.28738	
PI 321020	WR-242	0.89713	

PI 321020	WR-242	0.36380	
PI 321020	WR-242	0.62520	
PI 339545	WR-243	0.28782	
PI 339545	WR-243	0.36380	0.23948
PI 339545	WR-243	0.06681	
PI 339546	WR-244	0.03592	
PI 339546	WR-244	0.12871	
PI 339546	WR-244	0.17483	0.11945
PI 339546	WR-244	0.22639	
PI 339546	WR-244	0.03141	
PI 339547	WR-245	0.37055	
PI 339547	WR-245	0.03198	0.15360
PI 339547	WR-245	0.05828	
PI 339548	WR-246	0.13030	0.22452
PI 339548	WR-246	0.31874	
PI 344085	WR-247	0.23310	
PI 344085	WR-247	0.14409	0.13626
PI 344085	WR-247	0.03159	
PI 358247	WR-248	0.13448	
PI 358247	WR-248	0.09653	0.16902
PI 358247	WR-248	0.27604	
PI 358248	WR-249	1.11361	
PI 358248	WR-249	0.34279	0.94709
PI 358248	WR-249	1.38485	
PI 358249	WR-250	0.22318	
PI 358249	WR-250	0.05521	0.12578
PI 358249	WR-250	0.09896	
PI 358250	WR-251	0.18731	
PI 358250	WR-251	0.42814	0.24753
PI 358250	WR-251	0.12715	
PI 358251	WR-252	0.10022	
PI 358251	WR-252	0.39185	0.15869
PI 358251	WR-252	0.11402	
PI 358251	WR-252	0.02866	
PI 358252	WR-253	0.22663	
PI 358252	WR-253	0.16288	0.16934
PI 358252	WR-253	0.11853	
PI 358253	WR-254	0.20062	0.40074
PI 358253	WR-254	0.11159	

PI 358253	WR-254	0.89002	
PI 358254	WR-255	0.44636	
PI 358254	WR-255	0.23090	0.23586
PI 358254	WR-255	0.03032	
PI 358255	WR-256	0.65952	
PI 358255	WR-256	0.17483	0.37013
PI 358255	WR-256	0.27604	
PI 358256	WR-257	0.14409	
PI 358256	WR-257	0.12638	0.17855
PI 358256	WR-257	0.26518	
PI 358257	WR-258	0.13535	
PI 358257	WR-258	0.19306	0.20606
PI 358257	WR-258	0.28977	
PI 358258	WR-259	0.46880	
PI 358258	WR-259	0.21702	0.55199
PI 358258	WR-259	0.97013	
PI 358259	WR-260	0.36171	
PI 358259	WR-260	0.06207	0.49759
PI 358259	WR-260	1.06899	
PI 358260	WR-261	0.87977	
PI 358260	WR-261	0.10851	0.39378
PI 358260	WR-261	0.19306	
PI 360710	WR-262	0.20044	
PI 360710	WR-262	0.11464	0.17047
PI 360710	WR-262	0.19633	
PI 360894	WR-263	0.46700	
PI 360894	WR-263	0.13986	0.41798
PI 360894	WR-263	0.64708	
PI 360895	WR-264	0.10490	
PI 360895	WR-264	0.11080	0.28169
PI 360895	WR-264	0.62937	
PI 361127	WR-265	0.15609	
PI 361127	WR-265	0.16597	0.32438
PI 361127	WR-265	0.65107	
PI 368824	WR-266	0.16919	
PI 368824	WR-266	0.26556	0.49268
PI 368824	WR-266	1.04331	
PI 368826	WR-267	0.62520	0.50897
PI 368826	WR-267	0.20703	

PI 368826	WR-267	0.69467	
PI 370602	WR-268	0.14650	
PI 370602	WR-268	0.06099	0.29033
PI 370602	WR-268	0.66349	
PI 374233	WR-269	0.03406	
PI 374233	WR-269	0.03406	0.10016
PI 374233	WR-269	0.23236	
PI 379546	WR-270	0.27604	
PI 379546	WR-270	0.51482	
PI 379546	WR-270	0.03278	0.28040
PI 379546	WR-270	0.12871	
PI 379546	WR-270	0.08598	
PI 379546	WR-270	0.64409	
PI 379547	WR-271	0.14879	
PI 379547	WR-271	0.35613	0.29171
PI 379547	WR-271	0.37022	
PI 379548	WR-272	0.25674	
PI 379548	WR-272	0.23236	0.26016
PI 379548	WR-272	0.29138	
PI 379549	WR-273	0.20302	
PI 379549	WR-273	0.16088	0.32509
PI 379549	WR-273	0.61135	
PI 379550	WR-274	0.21222	
PI 379550	WR-274	0.05926	0.15565
PI 379550	WR-274	0.19546	
PI 379551	WR-275	0.14569	
PI 379551	WR-275	0.24806	0.16669
PI 379551	WR-275	0.10631	
PI 418978	WR-276	0.21853	
PI 418978	WR-276	0.32230	0.22032
PI 418978	WR-276	0.16562	
PI 418978	WR-276	0.17483	
PI 419004	WR-277	0.65559	
PI 419004	WR-277	0.14175	0.36684
PI 419004	WR-277	0.30317	
PI 419162	WR-278	0.06767	
PI 419162	WR-278	0.11320	0.12464
PI 419162	WR-278	0.03238	
PI 419162	WR-278	0.14879	

PI 419162	WR-278	0.17719	
PI 419162	WR-278	0.20860	
PI 419218	WR-279	0.49280	
PI 419218	WR-279	0.19668	0.26456
PI 419218	WR-279	0.10420	
PI 433207	WR-280	0.63235	
PI 433207	WR-280	0.09594	0.25383
PI 433207	WR-280	0.03319	
PI 433209	WR-281	0.33241	
PI 433209	WR-281	0.30651	0.27410
PI 433209	WR-281	0.18338	
PI 433210	WR-282	0.21554	0.25436
PI 433210	WR-282	0.29319	
PI 433211	WR-283	0.19188	
PI 433211	WR-283	0.03720	0.17722
PI 433211	WR-283	0.30258	
PI 433212	WR-284	0.20302	
PI 433212	WR-284	0.33937	0.23612
PI 433212	WR-284	0.16597	
PI 445782	WR-285	0.20840	0.12216
PI 445782	WR-285	0.03592	
PI 445785	WR-286	0.11340	
PI 445785	WR-286	0.20742	0.15619
PI 445785	WR-286	0.14774	
PI 449353	WR-287	0.39872	
PI 449353	WR-287	0.28435	0.23719
PI 449353	WR-287	0.02850	
PI 478393	WR-288	0.74366	
PI 478393	WR-288	0.20742	0.48300
PI 478393	WR-288	0.49792	
PI 499373	WR-290	0.19633	0.19633
PI 508504	WR-291	0.24681	
PI 508504	WR-291	0.28937	0.24230
PI 508504	WR-291	0.19072	
PI 531450	WR-292	0.31547	0.20295
PI 531450	WR-292	0.09043	
PI 531451	WR-293	0.06244	0.04741
PI 531451	WR-293	0.03238	
PI 531452	WR-294	0.13802	0.14055

PI 531452	WR-294	0.14468	
PI 531452	WR-294	0.13893	
PI 531453	WR-295	0.17719	
PI 531453	WR-295	0.23996	0.23550
PI 531453	WR-295	0.28937	
PI 531454	WR-296	0.07439	
PI 531454	WR-296	0.07040	0.05886
PI 531454	WR-296	0.03179	
PI 531455	WR-297	0.31219	
PI 531455	WR-297	0.12197	0.27827
PI 531455	WR-297	0.40064	
PI 531457	WR-298	0.21853	
PI 531457	WR-298	0.20044	0.23738
PI 531457	WR-298	0.29319	
PI 535897	WR-299	0.72668	
PI 535897	WR-299	0.15246	0.86066
PI 535897	WR-299	1.70284	
PI 604778	WR-300	0.37258	
PI 604778	WR-300	0.43706	0.41644
PI 604778	WR-300	0.43968	
PI 604779	WR-301	0.28350	
PI 604779	WR-301	0.06597	0.61460
PI 604779	WR-301	1.49432	
PI 604780	WR-302	0.30671	
PI 604780	WR-302	0.31185	0.22804
PI 604780	WR-302	0.06556	
PI 604782	WR-303	0.10631	
PI 604782	WR-303	0.40124	0.22912
PI 604782	WR-303	0.17982	
PI 604783	WR-304	0.03642	0.03642
PI 604784	WR-305	0.19668	
PI 604784	WR-305	0.11219	0.16222
PI 604784	WR-305	0.17779	
PI 604785	WR-306	0.06993	0.09156
PI 604785	WR-306	0.11320	
PI 604787	WR-307	0.03856	0.03856
PI 604788	WR-308	0.05640	
PI 604788	WR-308	0.24394	0.11234
PI 604788	WR-308	0.03668	

PI 604789	WR-309	0.14468	
PI 604789	WR-309	0.14175	0.13068
PI 604789	WR-309	0.10560	
PI 604790	WR-310	0.15517	
PI 604790	WR-310	0.23705	0.19408
PI 604790	WR-310	0.19003	
PI 604791	WR-311	0.22083	
PI 604791	WR-311	0.38462	0.25416
PI 604791	WR-311	0.15703	
PI 647852	WR-312	0.03406	0.18093
PI 647852	WR-312	0.32780	
PI 647853	WR-313	0.21853	
PI 647853	WR-313	0.14468	0.13321
PI 647853	WR-313	0.03642	
PI 647854	WR-314	0.06947	
PI 647854	WR-314	0.03122	0.05425
PI 647854	WR-314	0.06207	
PI 647855	WR-315	0.16493	0.11021
PI 647855	WR-315	0.05550	
PI 647856	WR-316	0.89197	
PI 647856	WR-316	1.05624	0.87691
PI 647856	WR-316	0.68254	
PI 647857	WR-317	1.13400	1.13400
PI 647858	WR-318	0.51347	0.82374
PI 647858	WR-318	1.13400	
PI 648936	WR-319	1.25041	
PI 648936	WR-319	1.32011	1.32617
PI 648936	WR-319	1.40799	
PI 648937	WR-320	1.21567	1.12518
PI 648937	WR-320	1.03468	
PI 648938	WR-321	1.17739	
PI 648938	WR-321	1.07674	1.18844
PI 648938	WR-321	1.31119	
PI 648940	WR-322	2.38060	
PI 648940	WR-322	2.65782	2.12236
PI 648940	WR-322	1.32867	
PI 648941	WR-323	0.94330	
PI 648941	WR-323	1.42358	1.03702
PI 648941	WR-323	0.74419	

PI 648942	WR-324	0.79237	
PI 648942	WR-324	1.14498	0.89376
PI 648942	WR-324	0.74394	
PI 648943	WR-325	1.32036	
PI 648943	WR-325	2.06294	1.49150
PI 648943	WR-325	1.09119	
PI 648945	WR-326	1.47155	
PI 648945	WR-326	1.89394	1.36823
PI 648945	WR-326	0.73919	
PI 648947	WR-327	1.25041	
PI 648947	WR-327	2.42344	1.44164
PI 648947	WR-327	0.65107	
PI 648948	WR-328	1.70194	
PI 648948	WR-328	1.41750	1.55608
PI 648948	WR-328	1.54879	
PI 648950	WR-329	0.50991	0.62692
PI 648950	WR-329	0.74394	
PI 648951	WR-330	0.90427	0.82173
PI 648951	WR-330	0.73919	
PI 648952	WR-331	0.70875	
PI 648952	WR-331	0.90909	0.70692
PI 648952	WR-331	0.50292	
PI 648953	WR-332	1.26598	
PI 648953	WR-332	0.52800	0.82395
PI 648953	WR-332	0.67789	
PI 648954	WR-333	0.78493	0.96255
PI 648954	WR-333	1.14016	
PI 648955	WR-334	1.23626	1.43197
PI 648955	WR-334	1.62768	
PI 648956	WR-335	0.97902	
PI 648956	WR-335	1.19679	0.96476
PI 648956	WR-335	0.71846	
PI 648958	WR-336	1.38206	1.58911
PI 648958	WR-336	1.79615	
PI 648959	WR-337	0.88594	
PI 648959	WR-337	1.50877	1.33437
PI 648959	WR-337	1.60839	
PI 648961	WR-338	1.32011	0.96662
PI 648961	WR-338	0.62937	

PI 648961	WR-338	0.95039	
PI 648962	WR-339	0.15158	
PI 648962	WR-339	0.18296	0.13452
PI 648962	WR-339	0.06901	
PI 648963	WR-340	1.08392	
PI 648963	WR-340	1.37279	1.21850
PI 648963	WR-340	1.19880	
PI 648964	WR-341	0.95681	1.12948
PI 648964	WR-341	1.30215	
PI 664497	WR-343	0.77439	
PI 664497	WR-343	0.80959	1.20399
PI 664497	WR-343	2.02797	
PI 664498	WR-344	1.83207	1.83207
PI 664499	WR-345	1.15385	
PI 664499	WR-345	1.78322	1.47182
PI 664499	WR-345	1.47839	
PI 677108	WR-346	1.00584	1.00584
PI 608712	WR-347	0.79237	1.04280
PI 608712	WR-347	1.29323	
PI 604792	WR-348	1.26469	
PI 604792	WR-348	1.55925	1.29569
PI 604792	WR-348	1.06313	
APRV 1	WR-349	1.34865	
APRV 1	WR-349	1.93984	1.48078
APRV 1	WR-349	1.15385	
APRV 2	WR-350	1.78393	1.69616
APRV 2	WR-350	1.60839	
APRV 4	WR-352	1.41066	1.74296
APRV 4	WR-352	2.07526	
APRV 5	WR-353	1.59151	1.71811
APRV 5	WR-353	1.84471	
APRV 6	WR-354	1.19030	
APRV 6	WR-354	0.73353	0.98600
APRV 6	WR-354	1.03418	
APRV 7	WR-355	1.46853	
APRV 7	WR-355	1.62768	1.50887
APRV 7	WR-355	1.43039	
APRV 8	WR-356	1.66556	1.53208
APRV 8	WR-356	1.39860	

APRV 9	WR-357	1.04895	1.04895
APRV 10	WR-358	1.60474	1.69471
APRV 10	WR-358	1.78467	
APRV 12	WR-360	1.43606	1.56177
APRV 12	WR-360	1.85315	
APRV 12	WR-360	1.39609	

Supplemental table 2. Ascorbic acid content significantly associated markers allele effects by GWAS model

						GLM				cMLM (P3D)				cMLM (permarker)				
Marker	Chr	Position	Alleles		Major allele freq	p-value	Allele with major effect	Effect	R2	p-value	Allele with major effect	Effect	R2	p-value	Allele with major effect	Effect	R2	h2
18446_91	1	26,086,756	C	T	0.94	6.56E-04	C	-0.45	0.04									
18447_135	1	26,224,404	C	A	0.94	6.56E-04	C	-0.45	0.04									
18997_127	1	32,069,169	T	G	0.70	9.31E-04	G	0.54	0.05									
19022_139	1	32,206,058	T	C	0.70	9.31E-04	C	0.54	0.05									
19027_0	1	32,243,743	A	T	0.69	4.53E-05	T	0.61	0.07									
19031_147	1	32,253,488	G	T	0.69	4.02E-04	T	0.54	0.06									
19031_71	1	32,253,412	A	G	0.69	4.02E-04	G	0.54	0.06									
19032_111	1	32,253,488	G	T	0.68	5.50E-05	T	0.61	0.07									
19037_84	1	32,307,120	C	A	0.68	5.50E-05	A	0.61	0.07									
19355_0	1	36,206,961	T	C	0.78	2.09E-03	T	-0.25	0.04									
19355_71	1	36,207,039	T	C	0.78	2.09E-03	T	-0.25	0.04									
19513_51	1	37,511,412	G	A	0.77	1.02E-03	G	-0.27	0.04									
19514_17	1	37,511,842	A	T	0.77	1.02E-03	A	-0.27	0.04									
19819_142	1	39,795,591	T	C	0.79	9.73E-04	T	-0.28	0.04									
19832_42	1	39,863,270	G	T	0.79	9.73E-04	G	-0.28	0.04									
19968_37	1	40,913,370	C	G	0.81	2.34E-05	G	0.82	0.08									
19968_80	1	40,913,413	C	T	0.81	2.34E-05	T	0.82	0.08									
20013_59	1	41,230,635	G	A	0.66	6.01E-04	G	0.30	0.05									
20014_86	1	41,230,635	G	A	0.66	6.01E-04	G	0.30	0.05									
20248_143	1	43,534,058	T	G	0.84	2.13E-04	G	0.77	0.06									
20250_0	1	43,534,176	T	G	0.84	2.13E-04	G	0.77	0.06									

21099_124	1	49,342,208	T	G	0.92	4.61E-04	G	1.27	0.06
21099_147	1	49,342,231	T	G	0.92	4.61E-04	G	1.27	0.06
21115_131	1	49,380,455	C	A	0.92	3.15E-04	A	1.26	0.06
21115_133	1	49,380,453	C	G	0.92	3.15E-04	G	1.26	0.06
21255_99	1	50,390,815	G	T	0.72	5.40E-07	T	0.82	0.12
21256_74	1	50,390,815	G	T	0.72	5.40E-07	T	0.82	0.12
21292_120	1	5,140,768	T	A	0.59	9.22E-04	A	0.54	0.05
21292_132	1	5,140,780	G	A	0.82	5.88E-04	A	0.78	0.06
21292_98	1	5,140,746	C	T	0.59	9.22E-04	T	0.54	0.05
21294_108	1	5,140,822	C	G	0.82	5.88E-04	G	0.78	0.06
21472_119	1	7,236,734	G	T	0.90	1.25E-04	T	0.94	0.07
21473_74	1	7,236,734	G	T	0.90	1.25E-04	T	0.94	0.07
21500_21	1	7,433,915	A	G	0.92	3.29E-05	G	0.89	0.08
21513_40	1	7,907,820	A	G	0.66	6.30E-04	G	0.76	0.06
21522_144	1	7,976,203	A	C	0.66	6.30E-04	C	0.76	0.06
22329_69	2	25,391,528	G	A	0.92	5.16E-04	G	-0.13	0.06
22449_54	2	28,231,032	C	T	0.94	1.84E-07	C	-0.64	0.10
22452_66	2	28,293,382	G	A	0.94	1.84E-07	G	-0.64	0.10
22571_88	2	30,189,324	C	T	0.81	1.51E-03	T	2.25	0.06
22576_7	2	30,257,405	T	C	0.81	1.51E-03	C	2.25	0.06
22578_49	2	30,280,193	C	A	0.84	1.68E-03	A	2.27	0.06
22594_0	2	30,388,094	T	A	0.84	1.68E-03	A	2.27	0.06
22884_92	2	35,733,419	A	T	0.51	6.37E-04	A	0.47	0.06
22884_98	2	35,733,425	T	A	0.51	6.37E-04	T	0.47	0.06
22885_109	2	35,733,738	C	T	0.95	1.27E-08	C	-0.76	0.12
22885_111	2	35,733,740	C	T	0.95	1.27E-08	C	-0.76	0.12
23191_35	2	40,173,581	G	T	0.75	8.69E-05	T	0.82	0.07

23195_145	2	40,183,489	G	C	0.75	8.69E-05	C	0.82	0.07
23199_95	2	40,231,531	T	G	0.75	8.54E-05	G	0.82	0.07
23213_66	2	40,459,411	T	A	0.75	8.54E-05	A	0.82	0.07
23225_86	2	40,605,455	T	C	0.88	1.04E-05	T	-0.44	0.08
23233_143	2	40,671,312	T	G	0.88	1.04E-05	T	-0.44	0.08
23424_75	2	42,700,972	T	G	0.86	2.90E-04	G	0.96	0.06
23431_96	2	42,735,301	T	A	0.86	2.90E-04	A	0.96	0.06
23432_145	2	42,747,240	C	G	0.81	2.11E-03	G	0.73	0.05
23448_95	2	42,872,793	C	T	0.81	2.11E-03	T	0.73	0.05
23601_0	2	4,504,336	T	A	0.94	1.46E-03	A	-0.61	0.05
23637_44	2	4,549,925	G	C	0.94	1.46E-03	C	-0.61	0.05
24058_64	2	49,652,698	G	A	0.94	2.66E-07	G	-0.63	0.10
24058_66	2	49,652,696	C	T	0.94	2.66E-07	C	-0.63	0.10
24704_131	2	54,129,556	A	G	0.93	1.74E-03	A	-0.37	0.04
24704_77	2	54,129,502	T	A	0.64	4.39E-05	A	0.63	0.07
24705_147	2	54,129,556	A	G	0.93	1.74E-03	A	-0.37	0.04
24962_121	2	55,727,156	C	T	0.86	3.53E-04	C	-0.32	0.05
24963_109	2	55,727,077	C	T	0.86	3.53E-04	C	-0.32	0.05
25316_140	2	58,214,345	T	A	0.94	1.93E-03	T	-0.40	0.04
25316_144	2	58,214,349	T	A	0.94	1.93E-03	T	-0.40	0.04
25648_103	2	60,396,323	T	C	0.94	7.02E-07	T	-0.61	0.09
25650_46	2	60,431,729	T	C	0.94	7.02E-07	T	-0.61	0.09
26394_140	3	11,201,518	G	C	0.80	2.00E-04	G	-0.34	0.06
26394_71	3	11,201,449	T	G	0.80	2.00E-04	T	-0.34	0.06
27513_96	3	2,579,672	A	G	0.80	8.06E-05	G	0.82	0.07
27514_54	3	2,579,672	A	G	0.80	8.06E-05	G	0.82	0.07
27590_0	3	2,689,687	A	T	0.81	2.21E-05	T	0.80	0.08

27687_0	3	2,808,515	A	T	0.94	1.47E-04	A	-0.47	0.06
27688_32	3	2,808,921	C	G	0.94	1.47E-04	C	-0.47	0.06
27858_148	3	3,148,769	A	G	0.72	2.22E-04	G	0.67	0.07
27964_22	3	3,406,045	A	G	0.80	3.10E-05	G	0.83	0.08
28020_33	3	3,540,253	C	T	0.74	6.60E-05	T	0.68	0.07
28077_143	3	3,688,025	C	G	0.74	6.60E-05	G	0.68	0.07
28108_149	3	3,743,058	C	T	0.74	6.07E-05	T	0.69	0.08
28117_142	3	3,745,218	G	T	0.74	6.07E-05	T	0.69	0.08
28177_11	3	3,853,344	C	G	0.74	6.60E-05	G	0.68	0.07
28185_72	3	3,870,900	A	G	0.74	6.60E-05	G	0.68	0.07
28250_142	3	3,943,167	C	G	0.74	6.24E-05	G	0.69	0.07
28250_75	3	3,943,100	C	T	0.74	6.24E-05	T	0.69	0.07
28263_78	3	3,956,975	G	A	0.74	3.90E-04	A	0.63	0.06
28263_92	3	3,956,961	T	A	0.74	3.90E-04	A	0.63	0.06
28549_88	3	4,427,955	G	T	0.56	2.00E-03	G	0.36	0.05
28550_27	3	4,428,115	G	A	0.56	2.00E-03	G	0.36	0.05
28937_41	3	51,955,976	C	T	0.65	9.11E-05	T	0.60	0.07
28939_73	3	51,962,072	A	G	0.65	9.11E-05	G	0.60	0.07
28939_75	3	51,962,074	C	G	0.66	7.39E-05	G	0.60	0.07
28947_0	3	51,994,924	A	T	0.66	7.39E-05	T	0.60	0.07
29531_45	3	6,270,145	C	T	0.65	6.80E-04	C	0.46	0.05
29532_136	3	6,270,145	C	T	0.65	6.80E-04	C	0.46	0.05
29555_0	3	6,322,399	A	G	0.65	1.16E-03	A	0.44	0.05
29557_0	3	6,334,215	A	T	0.65	1.16E-03	A	0.44	0.05
29573_53	3	6,361,232	G	T	0.65	6.33E-04	G	0.46	0.05
29583_0	3	6,386,626	T	A	0.65	6.33E-04	T	0.46	0.05
29583_92	3	6,386,683	T	C	0.65	6.33E-04	T	0.46	0.05

29649_30	3	6,475,814	A	T	0.65	6.33E-04	A	0.46	0.05
29706_149	3	655,179	A	G	0.66	1.52E-03	G	0.47	0.05
29947_8	3	699,742	T	C	0.66	1.52E-03	C	0.47	0.05
30158_28	3	74,370,145	T	G	0.95	4.76E-05	T	-0.55	0.07
30163_65	3	74,503,916	G	T	0.95	4.76E-05	G	-0.55	0.07
32481_143	4	115,303,744	G	A	0.78	7.76E-06	A	0.91	0.10
32519_92	4	115,809,492	C	T	0.92	1.04E-03	C	-0.38	0.05
32520_56	4	115,809,492	C	T	0.92	1.04E-03	C	-0.38	0.05
33681_148	4	18,084,042	T	G	0.94	1.34E-03	T	-0.42	0.04
33683_0	4	18,108,994	T	A	0.94	1.34E-03	T	-0.42	0.04
33757_0	4	19,145,345	T	C	0.90	8.30E-04	T	-0.34	0.04
33757_12	4	19,145,482	C	A	0.90	8.30E-04	C	-0.34	0.04
34733_43	4	47,437,830	G	A	0.71	5.89E-04	A	0.79	0.06
34738_120	4	47,544,863	G	C	0.71	5.89E-04	C	0.79	0.06
34883_38	4	51,195,861	T	C	0.57	2.06E-03	T	0.36	0.05
34901_12	4	51,448,355	C	T	0.57	2.06E-03	C	0.36	0.05
34913_24	4	51,557,723	C	T	0.84	1.81E-06	T	1.24	0.10
34989_118	4	5,333,134	T	C	0.94	1.70E-05	T	-0.52	0.07
34990_9	4	5,337,392	C	T	0.94	1.70E-05	C	-0.52	0.07
35087_0	4	55,154,629	A	T	0.83	8.68E-05	T	0.90	0.07
35093_1	4	55,155,496	A	C	0.83	8.68E-05	C	0.90	0.07
35965_128	4	7,461,531	G	A	0.95	5.10E-06	G	-0.58	0.08
35966_107	4	7,461,531	G	A	0.95	5.10E-06	G	-0.58	0.08
36247_148	4	78,781,464	A	C	0.94	1.27E-04	A	-0.46	0.06
36249_0	4	78,799,506	A	T	0.94	1.27E-04	A	-0.46	0.06
36675_142	4	85,449,268	T	G	0.95	8.32E-05	G	1.37	0.07
36678_0	4	85,593,100	A	T	0.95	8.32E-05	T	1.37	0.07

39454_145	5	37,592,017	G	T	0.83	2.47E-04	T	0.71	0.06
39454_146	5	37,592,018	G	C	0.83	2.47E-04	C	0.71	0.06
39469_0	5	37,826,724	T	A	0.84	5.48E-04	A	0.74	0.06
39491_1	5	39,065,637	A	C	0.84	5.48E-04	C	0.74	0.06
39497_65	5	39,158,874	G	A	0.84	1.18E-04	A	0.80	0.07
39497_8	5	39,158,817	G	A	0.84	1.18E-04	A	0.80	0.07
39497_93	5	39,158,902	A	G	0.85	1.50E-03	G	0.71	0.05
39553_111	5	40,016,036	T	G	0.87	1.09E-05	G	0.94	0.08
39553_133	5	40,016,058	C	T	0.70	1.31E-03	T	0.61	0.05
39553_80	5	40,016,005	C	T	0.85	1.50E-03	T	0.71	0.05
39554_44	5	40,016,218	A	T	0.70	1.31E-03	T	0.61	0.05
39973_67	5	47,389,521	A	G	0.89	5.37E-04	A	-0.35	0.05
39977_7	5	47,393,417	A	T	0.89	5.37E-04	A	-0.35	0.05
40570_125	5	64,568,044	G	A	0.94	9.20E-06	A	1.52	0.10
40570_132	5	64,568,051	C	A	0.94	9.20E-06	A	1.52	0.10
41044_24	5	9,280,921	T	A	0.94	9.00E-04	T	-0.43	0.04
41044_26	5	9,280,919	T	G	0.94	9.00E-04	T	-0.43	0.04
41320_124	6	14,602,008	T	C	0.94	1.70E-03	C	1.39	0.05
41320_140	6	14,602,024	T	C	0.94	1.70E-03	C	1.39	0.05
41588_88	6	18,864,209	C	G	0.80	1.18E-03	C	-0.28	0.04
41596_81	6	18,974,282	G	A	0.80	1.18E-03	G	-0.28	0.04
41833_30	6	21,987,025	T	C	0.94	4.37E-05	C	1.99	0.07
42736_61	6	32,402,383	C	T	0.91	1.71E-03	C	1.15	0.06
42739_0	6	32,403,159	A	T	0.91	1.71E-03	A	1.15	0.06
43614_13	6	39,692,322	A	G	0.90	4.63E-05	A	-0.40	0.06
43614_25	6	39,692,334	G	A	0.90	4.63E-05	G	-0.40	0.06
44214_102	6	44,765,221	T	C	0.94	6.46E-06	T	-0.58	0.08

44214_141	6	44,765,260	G	A	0.94	6.46E-06	G	-0.58	0.08				
44273_61	6	45,310,152	C	T	0.85	1.03E-05	T	0.77	0.10				
44277_51	6	45,336,145	C	T	0.85	1.03E-05	T	0.77	0.10				
44286_49	6	45,342,881	C	A	0.86	1.89E-05	C	0.70	0.09				
44286_64	6	45,342,866	T	C	0.85	1.50E-05	C	0.88	0.09				
17631_35	1	10,773,565	G	A	0.74					1.36E-03	A	1.95	0.34
17830_19	1	13,647,788	A	G	0.75					7.45E-05	G	2.07	0.99
17835_68	1	13,661,581	C	T	0.75					7.45E-05	T	2.07	0.99
18420_84	1	25,954,646	A	G	0.57					1.11E-03	G	0.63	0.09
18421_149	1	25,954,646	A	G	0.57					1.11E-03	G	0.63	0.09
18773_51	1	30,188,430	A	G	0.60					1.81E-06	A	1.60	0.46
18779_148	1	30,323,142	C	T	0.60					1.81E-06	C	1.60	0.46
18787_147	1	30,343,301	T	A	0.90					1.60E-04	A	2.89	0.25
18813_145	1	30,546,188	G	A	0.90					2.11E-05	A	3.63	0.57
18813_17	1	30,546,060	C	A	0.90					1.60E-04	A	2.89	0.25
18814_24	1	30,546,394	T	G	0.90					2.11E-05	G	3.63	0.57
19221_145	1	33,980,168	A	C	0.89					4.02E-05	C	3.28	0.63
19230_4	1	34,020,770	G	T	0.89					4.02E-05	T	3.28	0.63
19263_96	1	34,763,766	C	A	0.68					2.06E-05	A	-0.34	0.35
19263_99	1	34,763,769	C	A	0.68					2.06E-05	A	-0.34	0.35
19832_57	1	39,863,285	A	G	0.94					1.16E-05	A	1.72	0.16
19837_17	1	39,899,336	A	G	0.94					1.16E-05	A	1.72	0.16
20338_66	1	44,174,962	T	C	0.54					2.42E-05	C	1.64	0.84
21169_65	1	49,642,137	A	G	0.56					9.53E-11	A	0.34	0.03
21170_0	1	49,645,084	A	G	0.56					9.53E-11	A	0.34	0.03
21837_17	2	14,133,093	A	C	0.78					6.26E-05	C	-1.65	0.42
21837_20	2	14,133,090	A	C	0.78					6.26E-05	C	1.65	0.42

21892_32	2	16,614,443	G	A	0.73	2.25E-07	G	1.55	0.65
22554_113	2	29,993,544	G	C	0.63	7.28E-04	C	-1.10	0.73
22702_0	2	31,412,307	T	A	0.85	2.52E-04	A	-3.05	0.29
22786_0	2	34,859,308	A	T	0.85	1.97E-06	T	-3.67	NaN
22790_8	2	34,900,764	C	T	0.85	1.97E-06	T	-3.67	NaN
23824_53	2	47,802,396	G	C	0.94	9.96E-04	C	-6.31	0.76
23840_113	2	47,917,645	G	A	0.94	9.96E-04	A	-6.31	0.76
25069_41	2	56,471,027	G	A	0.85	1.58E-04	A	1.89	0.38
25069_62	2	56,471,048	T	C	0.85	1.58E-04	C	1.89	0.38
25430_32	2	58,860,729	C	G	0.90	6.29E-04	C	1.54	0.26
25431_111	2	58,860,940	A	T	0.87	1.96E-04	A	-0.17	0.03
25431_117	2	58,860,934	A	T	0.87	1.96E-04	A	-0.17	0.03
25431_148	2	58,860,903	G	T	0.87	1.96E-04	G	-0.17	0.03
26255_18	3	108,266,751	C	T	0.76	7.31E-04	C	1.00	0.32
26385_71	3	111,974,439	A	C	0.91	5.82E-07	C	-3.67	0.49
26565_46	3	12,403,753	A	C	0.64	2.29E-04	A	-0.65	0.20
26566_147	3	12,403,753	A	C	0.64	2.29E-04	A	-0.65	0.20
26876_1	3	16,064,491	A	C	0.57	2.25E-04	A	0.90	0.26
26884_0	3	16,105,912	A	C	0.57	2.25E-04	A	0.90	0.26
28237_70	3	39,199,668	A	G	0.58	1.67E-03	G	0.57	0.07
29430_102	3	60,678,645	C	G	0.80	7.21E-04	C	0.40	0.33
29481_132	3	61,755,981	G	T	0.80	7.21E-04	G	0.40	0.33
29526_47	3	62,474,252	A	T	0.80	2.84E-04	T	2.30	0.91
29529_92	3	62,540,090	A	G	0.80	2.84E-04	G	2.30	0.91
29971_66	3	70,498,159	A	G	0.78	1.14E-03	G	0.44	NaN
29973_136	3	70,498,159	A	G	0.78	1.14E-03	G	0.44	NaN
30083_39	3	72,559,560	A	G	0.90	3.27E-05	G	1.16	0.12

30083_6	3	72,559,593	T	A	0.90	3.27E-05	A	1.16	0.06
30151_12	3	743,510	G	A	0.91	1.09E-03	A	1.60	0.12
30156_137	3	743,510	G	A	0.91	1.09E-03	A	1.60	0.12
30205_109	3	7,568,163	G	A	0.76	1.50E-03	A	1.43	0.11
30205_57	3	7,568,111	G	A	0.86	1.51E-03	A	1.59	0.12
30206_67	3	7,568,422	A	T	0.76	2.64E-04	T	1.83	0.18
30671_114	3	8,620,811	A	G	0.92	1.10E-04	G	-3.33	0.38
30799_148	3	88,465,472	G	C	0.84	6.09E-04	C	1.80	0.45
30800_38	3	88,465,472	G	C	0.84	6.09E-04	C	1.80	0.45
31487_100	4	100,381,644	T	C	0.72	1.64E-03	C	0.99	0.08
31489_83	4	100,381,644	T	C	0.72	1.64E-03	C	0.99	0.08
31747_46	4	10,380,625	T	A	0.93	6.01E-93	A	2.19	0.16
31749_105	4	10,380,625	T	A	0.93	6.01E-93	A	-2.19	0.16
32854_93	4	118,696,771	C	T	0.91	2.26E-04	C	-1.62	0.64
33007_60	4	119,528,399	G	A	0.88	7.56E-24	G	-0.57	0.81
33559_113	4	16,421,490	A	T	0.69	1.28E-03	A	0.77	0.11
34035_132	4	26,810,902	G	C	0.84	3.04E-05	C	1.79	0.32
34036_132	4	26,818,569	T	C	0.84	3.04E-05	C	1.79	0.32
34039_42	4	26,819,040	T	C	0.74	7.95E-04	C	1.02	0.13
34039_87	4	26,818,995	T	A	0.74	7.95E-04	A	1.02	0.13
34117_137	4	28,325,117	G	A	0.53	1.44E-04	A	0.70	0.11
34118_143	4	28,325,294	C	T	0.53	2.15E-06	T	1.05	0.25
34242_136	4	31,387,745	A	C	0.91	1.40E-03	C	1.03	0.07
34271_61	4	32,550,978	C	T	0.76	2.52E-06	T	4.39	0.68
35060_72	4	54,436,791	T	C	0.60	9.25E-06	C	1.65	0.56
35061_75	4	54,436,791	T	C	0.60	9.25E-06	C	1.65	0.56
35072_118	4	54,716,214	C	T	0.78	2.65E-04	T	1.76	0.11

35073_91	4	54,716,214	C	T	0.78	2.65E-04	T	1.76	0.11
35144_0	4	5,610,249	A	G	0.82	8.18E-04	G	1.10	0.13
35192_136	4	5,687,454	A	G	0.82	8.18E-04	G	1.10	0.13
37772_125	5	12,402,600	T	G	0.90	6.01E-06	T	0.67	0.42
37773_10	5	12,402,647	T	G	0.90	6.01E-06	T	0.67	0.42
38605_0	5	22,806,525	A	T	0.82	1.45E-03	A	0.89	0.32
38677_87	5	2,430,492	C	T	0.84	6.69E-05	T	1.03	0.10
38765_60	5	253,906	G	T	0.55	1.37E-03	T	0.58	0.06
38846_0	5	2,660,648	A	T	0.84	6.69E-05	T	1.03	0.10
39017_113	5	2,866,480	A	C	0.94	3.29E-04	A	2.12	0.33
39133_63	5	303,261	G	A	0.55	7.98E-04	A	0.63	0.07
39624_108	5	41,089,616	G	A	0.62	1.52E-03	A	0.64	0.08
39626_18	5	41,090,038	T	C	0.62	1.52E-03	C	0.64	0.08
39928_138	5	45,792,763	C	T	0.84	2.62E-05	C	0.43	0.03
39928_29	5	45,792,872	C	T	0.84	2.62E-05	C	0.43	0.03
40321_114	5	54,817,921	C	A	0.85	3.43E-04	A	0.93	0.16
40625_79	5	674,700	C	T	0.50	1.39E-05	T	0.98	0.30
40735_0	5	694,638	A	T	0.50	1.39E-05	T	0.98	0.30
41177_60	6	11,745,075	G	A	0.93	3.37E-07	A	4.89	0.78
41186_0	6	12,079,129	A	T	0.93	3.17E-05	T	3.34	0.35
41452_59	6	17,027,317	A	T	0.58	1.29E-03	A	-0.96	0.30
41504_103	6	17,811,377	T	C	0.84	3.19E-04	T	0.32	0.02
41661_12	6	19,763,961	T	A	0.95	1.14E-03	A	-3.00	0.12
42094_0	6	25,976,357	A	T	0.55	1.09E-03	A	0.68	0.13
42099_16	6	25,978,488	C	T	0.55	1.09E-03	C	0.68	0.13
42181_69	6	2,643,950	G	A	0.55	1.35E-03	G	0.56	0.11
42252_95	6	2,698,203	T	G	0.55	1.35E-03	T	0.56	0.11

42409_119	6	28,347,889	A	C	0.56	1.62E-03	A	0.44	0.05					
42415_26	6	28,362,409	G	A	0.56	6.28E-04	G	0.51	0.07					
42715_147	6	32,370,490	C	A	0.91	1.40E-03	A	0.40	0.01					
42737_145	6	32,402,180	T	A	0.91	1.40E-03	A	0.40	0.01					
42767_70	6	32,519,350	A	G	0.93	1.98E-04	G	0.95	0.04					
42952_0	6	34,212,703	A	G	0.88	1.56E-03	G	1.21	0.08					
42959_132	6	34,218,828	A	C	0.88	1.56E-03	C	1.21	0.08					
43139_149	6	35,410,747	G	T	0.94	7.05E-04	T	1.31	0.06					
43229_0	6	36,336,517	A	G	0.86	1.56E-03	G	1.93	0.34					
43483_45	6	38,633,662	A	T	0.50	1.93E-04	A	1.07	0.36					
43489_42	6	38,651,061	C	G	0.50	1.93E-04	C	1.07	0.36					
43496_89	6	38,741,448	G	A	0.89	6.70E-06	A	0.48	0.05					
43500_132	6	38,755,116	C	T	0.89	8.56E-05	T	0.37	0.03					
43721_16	6	40,661,995	G	A	0.92	6.53E-13	A	-0.91	0.04					
43722_133	6	40,687,330	T	C	0.92	6.53E-13	C	-0.91	0.04					
43724_99	6	40,687,330	T	C	0.92	2.86E-17	C	3.45	0.38					
44122_1	6	43,822,462	T	G	0.93	1.23E-03	G	-0.21	0.02					
44132_19	6	43,909,231	T	C	0.93	1.23E-03	C	-0.21	0.02					
44154_56	6	44,253,924	T	C	0.91	3.46E-06	T	0.10	0.00					
44154_69	6	44,253,937	C	A	0.91	3.46E-06	C	0.10	0.00					
44175_144	6	44,490,636	A	G	0.91	4.41E-09	A	0.15	0.01					
44179_24	6	44,496,133	C	T	0.91	4.41E-09	C	0.15	0.01					
44474_125	6	7,040,639	T	C	0.91	6.24E-04	C	0.63	0.17					
44474_59	6	7,040,573	G	A	0.91	6.24E-04	A	0.63	0.17					
52966_144	6	45,522,356	G	T	0.91	7.22E-04	G	1.26	0.21					
18253_80	1	23,243,114	C	T	0.80					1.01E-04	T	1.27	0.32	0.79922
19686_116	1	38,745,477	C	T	0.94					7.78E-07	T	7.47	0.58	0.92852

19895_47	1	40,433,857	A	C	0.88	8.56E-04	C	1.63	0.11	0.93561
20428_123	1	44,730,648	C	G	0.52	1.65E-04	C	0.81	0.55	0.59643
20429_38	1	44,730,648	C	G	0.52	1.65E-04	C	0.81	0.55	0.59643
21406_95	1	64,715	G	A	0.81	8.32E-05	A	0.90	0.12	0.88194
22066_109	2	19,582,976	G	C	0.61	2.50E-04	C	0.79	0.09	0.89599
22068_147	2	19,582,866	T	A	0.61	2.50E-04	A	0.79	0.09	0.89599
23027_134	2	3,805,442	T	G	0.54	1.89E-27	T	0.17	0.03	0.84724
23134_58	2	39,622,079	A	C	0.67	3.59E-05	C	2.13	0.77	0.73463
23155_93	2	40,008,560	A	G	0.67	3.59E-05	G	2.13	0.77	0.73463
23937_86	2	48,648,516	T	G	0.84	1.26E-04	G	1.73	0.11	0.93829
23951_12	2	4,882,570	C	A	0.89	3.13E-04	A	-1.98	0.39	1.2429
23952_42	2	4,882,770	T	C	0.89	3.13E-04	C	-1.98	0.39	1.2429
23963_76	2	48,855,023	C	T	0.84	2.38E-04	T	2.03	0.15	0.93028
24275_0	2	50,997,283	T	A	0.71	8.45E-04	A	0.79	0.08	0.90555
24284_6	2	51,045,598	G	A	0.71	8.45E-04	A	0.79	0.08	0.90555
24289_62	2	51,133,139	G	A	0.70	2.72E-06	A	1.89	0.62	0.75234
24294_15	2	51,212,634	C	A	0.70	2.72E-06	A	1.89	0.62	0.75234
24484_0	2	52,272,488	T	A	0.63	6.88E-04	A	1.02	0.21	0.83155
24487_0	2	52,280,683	A	T	0.55	1.20E-03	A	0.52	0.07	0.87789
24511_8	2	52,364,078	G	T	0.55	1.20E-03	G	0.52	0.07	0.87789
24765_89	2	54,639,882	C	A	0.77	3.90E-05	A	1.52	0.45	0.77268
25127_28	2	56,656,947	T	C	0.65	9.55E-04	T	1.53	0.59	0.72347
25162_8	2	56,878,757	G	C	0.65	9.55E-04	G	1.53	0.59	0.72347
25431_75	2	58,860,976	T	C	0.87	1.04E-03	C	0.37	0.01	0.9647
25436_16	2	58,889,189	C	A	0.87	1.04E-03	A	0.37	0.01	0.9647
25436_24	2	58,889,197	G	A	0.87	1.04E-03	A	0.37	0.01	0.9647
25437_141	2	58,889,197	G	A	0.87	1.04E-03	A	0.37	0.01	0.9647

25437_149	2	58,889,189	C	A	0.87	1.04E-03	A	0.37	0.01	0.9647
26391_148	3	11,201,274	T	G	0.89	2.30E-28	G	-0.25	0.02	1.06523
26394_5	3	11,201,383	G	A	0.89	2.30E-28	A	-0.25	0.02	1.06523
26597_53	3	12,731,426	C	T	0.65	6.60E-05	C	0.50	0.13	0.79049
26599_101	3	12,731,426	C	T	0.65	6.60E-05	C	0.50	0.13	0.79049
26903_132	3	16,213,630	G	A	0.88	4.94E-04	G	0.94	0.16	0.85633
26906_9	3	16,220,233	G	C	0.88	4.94E-04	G	0.94	0.16	0.85633
31213_146	3	95,602,919	G	A	0.56	9.32E-05	A	1.36	0.48	0.73846
31213_80	3	95,602,853	G	C	0.56	9.32E-05	C	1.36	0.48	0.73846
31253_134	3	96,280,174	G	T	0.56	8.52E-04	T	0.62	0.07	0.89278
31253_135	3	96,280,175	G	T	0.56	8.52E-04	T	0.62	0.07	0.89278
31541_147	4	100,891,594	A	T	0.85	1.34E-11	T	-3.08	0.54	1.21082
33871_81	4	21,136,142	C	T	0.63	1.30E-04	C	1.67	0.95	0.63775
33962_87	4	25,149,405	C	G	0.61	4.17E-04	C	0.77	0.33	0.69836
33968_36	4	25,447,636	A	G	0.61	4.17E-04	A	0.77	0.33	0.69836
34242_83	4	31,387,798	G	A	0.91	4.28E-04	A	0.96	0.10	0.90225
34332_85	4	34,713,850	T	A	0.92	2.58E-05	A	2.73	0.38	0.87904
34498_36	4	40,261,080	A	G	0.86	8.05E-04	G	2.13	0.13	0.94216
34498_37	4	40,261,081	C	G	0.86	8.05E-04	G	2.13	0.13	0.94216
34762_104	4	47,845,972	T	C	0.74	2.63E-04	C	0.97	0.11	0.89825
36212_42	4	78,322,265	A	T	0.64	3.51E-04	T	1.40	0.50	0.73881
36553_140	4	84,239,999	G	C	0.89	4.53E-04	C	2.25	0.13	0.94611
36554_95	4	84,239,999	G	C	0.89	4.53E-04	C	2.25	0.13	0.94611
37433_86	4	98,770,437	G	T	0.91	2.42E-11	T	0.58	0.01	0.98195
37435_133	4	98,770,437	G	T	0.91	2.42E-11	T	0.58	0.01	0.98195
38885_75	5	2,676,049	G	T	0.84	1.49E-14	T	0.47	0.02	0.95722
38886_118	5	2,676,049	G	T	0.84	1.49E-14	T	0.47	0.02	0.95722

39759_146	5	42,767,871	T	C	0.93								4.33E-04	T	0.11	0.02	0.87845
40173_114	5	5,143,287	T	C	0.51								4.84E-04	C	0.94	0.31	0.75105
42189_110	6	26,519,627	G	A	0.57								1.13E-03	G	0.44	0.06	0.87424
42416_5	6	28,362,655	A	G	0.56								3.01E-04	A	0.52	0.11	0.82598
42423_18	6	28,430,761	C	T	0.56								3.01E-04	C	0.52	0.11	0.82598
42767_64	6	32,519,356	C	T	0.93								5.92E-04	T	1.81	0.14	0.92891
42769_96	6	32,540,672	G	T	0.93								5.92E-04	T	1.81	0.14	0.92891
43458_141	6	38,350,300	C	T	0.65								5.24E-04	C	0.65	0.14	0.81913
43458_38	6	38,350,197	A	G	0.65								5.24E-04	A	0.65	0.14	0.81913
43636_80	6	39,917,492	G	A	0.91								9.16E-04	A	0.92	0.04	0.96076
43739_4	6	40,798,438	A	G	0.79								1.08E-03	G	1.08	0.12	0.89966
43829_58	6	41,499,476	A	T	0.92								8.55E-04	A	0.33	0.03	0.91188
43831_125	6	41,505,741	A	T	0.92								8.55E-04	A	0.33	0.03	0.91188
44061_0	6	43,411,046	A	T	0.87								5.37E-07	T	2.29	0.65	0.77816
44066_80	6	43,426,953	C	T	0.87								5.37E-07	T	2.29	0.65	0.77816
44093_13	6	43,539,878	A	G	0.71								4.29E-04	G	0.75	0.10	0.88677
44095_138	6	43,540,150	A	T	0.71								4.29E-04	T	0.75	0.10	0.88677
44185_68	6	44,519,636	G	A	0.96								2.45E-04	A	2.61	0.11	0.95925
44190_115	6	44,602,354	G	A	0.96								2.45E-04	A	2.61	0.11	0.95925
44257_91	6	45,185,282	A	C	0.86								1.37E-06	C	-3.33	0.77	1.30312
44258_61	6	45,185,282	A	C	0.86								1.37E-06	C	-3.33	0.77	1.30312
50006_58	3	1,999,745	A	G	0.55								7.86E-04	G	0.50	0.07	0.87159
20279_125	1	43,647,136	T	A	0.85	2.05E-05	A	0.81	0.08	1.74E-04	A	1.06	0.07				
20279_78	1	43,647,089	G	A	0.85	2.05E-05	A	0.81	0.08	1.74E-04	A	1.06	0.07				
20965_149	1	48,593,828	T	C	0.94	5.10E-05	C	0.95	0.07	1.26E-08	C	2.76	0.55				
20970_7	1	48,610,543	G	A	0.94	5.10E-05	A	0.95	0.07	1.26E-08	A	2.76	0.55				
21101_74	1	49,342,286	C	T	0.92	4.61E-04	T	1.13	0.06	1.46E-09	T	3.38	0.79				

21113_147	1	49,380,453	C	G	0.92	4.61E-04	G	1.13	0.06	1.46E-09	G	3.38	0.79
21250_98	1	50,330,048	C	G	0.72	1.42E-07	G	0.80	0.12	1.97E-06	G	0.99	0.18
21252_144	1	50,330,048	C	G	0.72	1.42E-07	G	0.80	0.12	1.97E-06	G	0.99	0.18
21464_139	1	7,152,451	G	A	0.91	1.20E-04	A	0.59	0.08	3.37E-05	A	1.94	0.44
21464_77	1	7,152,389	G	A	0.65	2.50E-05	A	0.57	0.08	1.50E-03	A	0.47	0.05
21465_15	1	7,152,451	G	A	0.91	1.20E-04	A	0.59	0.08	3.37E-05	A	1.94	0.44
21465_77	1	7,152,389	G	A	0.65	2.50E-05	A	0.57	0.08	1.50E-03	A	0.47	0.05
21854_52	2	14,931,082	C	T	0.63	2.46E-05	T	0.52	0.08	4.63E-08	T	1.49	0.42
22327_141	2	25,043,877	G	A	0.56	1.86E-03	G	0.34	0.05	1.16E-04	G	0.60	0.14
22327_145	2	25,043,873	A	T	0.56	1.86E-03	A	0.34	0.05	1.16E-04	A	0.60	0.14
23442_135	2	42,838,538	C	G	0.86	2.97E-04	G	0.89	0.06	1.45E-07	G	2.14	0.31
23446_72	2	42,872,697	A	G	0.86	2.97E-04	G	0.89	0.06	1.45E-07	G	2.14	0.31
24704_113	2	54,129,538	A	G	0.64	4.39E-05	G	0.55	0.07	1.33E-06	G	1.26	0.30
27548_30	3	2,604,203	T	G	0.81	2.21E-05	G	0.68	0.08	1.02E-03	G	0.86	0.07
27616_18	3	2,712,980	A	C	0.90	3.35E-08	C	1.01	0.13	2.41E-04	C	0.85	0.08
27657_47	3	2,759,377	A	T	0.90	3.35E-08	T	1.01	0.13	2.41E-04	T	0.85	0.08
27876_41	3	3,192,000	T	C	0.72	2.22E-04	C	0.57	0.07	4.61E-04	C	1.01	0.13
27964_0	3	3,406,023	A	G	0.91	1.15E-12	G	1.62	0.21	4.04E-05	G	1.04	0.06
28308_146	3	40,243,417	T	C	0.92	1.07E-03	C	1.25	0.05	1.22E-05	C	3.00	0.27
28906_0	3	51,645,416	A	G	0.76	1.65E-05	G	0.71	0.08	1.58E-04	G	0.84	0.08
31356_46	3	97,879,214	C	G	0.94	1.63E-08	G	1.91	0.13	6.11E-05	G	6.64	0.32
31357_99	3	97,879,214	C	G	0.94	1.63E-08	G	1.91	0.13	6.11E-05	G	6.64	0.32
32486_135	4	115,313,459	A	G	0.79	6.52E-05	G	0.73	0.08	9.27E-04	G	1.04	0.10
32487_78	4	115,313,459	A	G	0.79	6.52E-05	G	0.73	0.08	9.27E-04	G	1.04	0.10
34907_53	4	51,498,162	G	C	0.84	1.81E-06	C	1.08	0.10	8.80E-04	C	0.94	0.06
34921_27	4	51,576,458	G	A	0.84	2.04E-07	A	1.29	0.12	1.50E-04	A	1.16	0.07
35053_25	4	5,432,524	C	T	0.81	4.07E-04	T	0.72	0.06	7.08E-07	T	1.50	0.24

35127_141	4	5,581,079	A	C	0.81	4.07E-04	C	0.72	0.06	7.08E-07	C	1.50	0.24					
35926_33	4	74,211,414	A	C	0.77	3.05E-06	C	0.91	0.10	1.51E-07	C	1.55	0.36					
35926_79	4	74,211,460	C	T	0.77	3.05E-06	T	0.91	0.10	1.51E-07	T	1.55	0.36					
40217_111	5	5,266,118	T	G	0.58	1.06E-03	T	0.29	0.05	4.55E-04	T	0.78	0.14					
40217_119	5	5,266,126	T	G	0.58	1.06E-03	T	0.29	0.05	4.55E-04	T	0.78	0.14					
40599_0	5	65,931,815	A	G	0.92	8.49E-05	G	1.04	0.07	1.33E-03	G	0.96	0.06					
40651_37	5	67,866,717	C	T	0.92	8.49E-05	T	1.04	0.07	1.33E-03	T	0.96	0.06					
41833_33	6	21,987,022	C	T	0.94	4.37E-05	T	1.80	0.07	5.38E-04	T	1.94	0.07					
42716_59	6	32,370,727	T	C	0.91	1.72E-03	T	0.68	0.06	4.83E-09	T	0.56	0.02					
42718_109	6	32,370,727	T	C	0.91	1.72E-03	T	0.68	0.06	4.83E-09	T	0.56	0.02					
44281_23	6	45,342,253	G	T	0.85	1.50E-05	T	0.54	0.09	6.27E-04	T	0.95	0.09					
19026_0	1	32,220,045	T	A	0.69	4.53E-05	A	0.61	0.07					5.20E-05	A	0.60	0.07	0.89258
19553_50	1	37,742,824	A	G	0.91	1.76E-04	G	1.00	0.06					8.72E-05	G	1.15	0.08	0.93495
19968_75	1	40,913,408	G	A	0.81	2.34E-05	A	0.82	0.08					1.54E-07	A	1.35	0.25	0.84404
19969_15	1	40,913,321	T	A	0.81	2.34E-05	A	0.82	0.08					1.54E-07	A	1.35	0.25	0.84404
19970_45	1	40,913,490	G	A	0.56	1.78E-03	A	0.49	0.05					8.80E-05	A	0.76	0.13	0.85745
19970_58	1	40,913,477	G	A	0.56	1.78E-03	A	0.49	0.05					8.80E-05	A	0.76	0.13	0.85745
23185_98	2	40,083,391	T	A	0.74	4.32E-04	A	0.71	0.06					3.39E-04	A	1.00	0.12	0.89045
23187_99	2	40,083,391	T	A	0.74	4.32E-04	A	0.71	0.06					3.39E-04	A	1.00	0.12	0.89045
23196_117	2	40,183,547	A	G	0.74	4.83E-04	G	0.70	0.06					5.65E-05	G	0.91	0.10	0.90176
23196_60	2	40,183,604	G	A	0.74	4.83E-04	A	0.70	0.06					1.70E-04	A	0.80	0.08	0.91237
23430_93	2	42,735,301	T	A	0.81	2.11E-03	A	0.73	0.05					3.65E-04	A	1.03	0.09	0.91633
23453_18	2	44,088,756	G	C	0.81	2.11E-03	C	0.73	0.05					3.65E-04	C	1.03	0.09	0.91633
27890_4	3	3,255,946	T	C	0.91	1.15E-12	C	1.79	0.21					2.98E-05	C	0.77	0.04	0.94789
27964_27	3	3,406,050	G	A	0.80	3.10E-05	A	0.83	0.08					1.28E-03	A	0.50	0.03	0.93604
28266_112	3	3,962,960	A	T	0.74	6.40E-05	T	0.69	0.07					1.59E-06	T	0.95	0.16	0.85812
28266_79	3	3,962,927	A	G	0.74	6.40E-05	G	0.69	0.07					1.59E-06	G	0.95	0.16	0.85812

28280_119	3	4,003,122	C	G	0.75	4.88E-05	G	0.75	0.08		1.60E-09	G	1.81	0.50	0.78318
28280_144	3	4,003,097	T	C	0.75	4.88E-05	C	0.75	0.08		9.12E-05	C	0.66	0.07	0.90614
28308_0	3	40,243,271	A	T	0.92	1.07E-03	T	1.25	0.05		5.73E-07	T	1.47	0.08	0.94876
31361_95	3	97,879,861	A	C	0.75	9.01E-04	C	0.57	0.06		1.05E-03	C	0.56	0.05	0.91133
31364_144	3	97,914,322	G	T	0.75	9.01E-04	T	0.57	0.06		1.05E-03	T	0.56	0.05	0.91133
32481_142	4	115,303,743	A	C	0.78	7.76E-06	C	0.91	0.10		9.80E-07	C	1.02	0.14	0.87901
33279_54	4	122,232,961	G	A	0.86	5.52E-05	A	1.00	0.07		1.00E-04	A	0.92	0.07	0.93389
33294_0	4	122,384,809	A	T	0.86	5.52E-05	T	1.00	0.07		5.68E-05	T	0.98	0.07	0.92959
34683_81	4	46,896,647	C	A	0.63	9.77E-04	A	0.66	0.05		8.22E-05	A	0.91	0.11	0.89089
34683_82	4	46,896,646	A	G	0.63	9.77E-04	G	0.66	0.05		9.87E-05	G	0.88	0.10	0.89802
34999_144	4	53,489,507	C	A	0.86	4.52E-07	A	1.17	0.11		5.64E-05	A	1.04	0.08	0.92529
35000_107	4	53,489,476	C	G	0.86	4.52E-07	G	1.17	0.11		2.03E-06	G	1.06	0.09	0.92511
35000_76	4	53,489,507	C	A	0.87	1.14E-05	A	1.03	0.08		5.28E-10	A	2.98	0.64	0.82217
35023_20	4	53,780,508	T	C	0.87	1.14E-05	C	1.03	0.08		5.28E-10	C	2.98	0.64	0.82217
35573_123	4	65,313,687	G	A	0.53	1.32E-04	A	0.49	0.07		2.41E-05	A	0.59	0.10	0.85727
35912_34	4	73,805,544	A	C	0.77	2.43E-06	C	0.98	0.10		7.29E-06	C	1.05	0.10	0.91334
35913_112	4	73,805,544	A	C	0.77	2.43E-06	C	0.98	0.10		7.29E-06	C	1.05	0.10	0.91334
39285_20	5	3,359,022	C	A	0.80	2.41E-04	A	0.75	0.06		9.93E-04	A	0.73	0.06	0.92623
39553_130	5	40,016,055	A	G	0.87	1.09E-05	G	0.94	0.08		4.14E-07	G	1.49	0.20	0.88076
40217_108	5	5,266,115	T	G	0.58	1.08E-03	T	0.43	0.05		6.66E-04	T	0.46	0.06	0.88293
40217_110	5	5,266,117	T	G	0.58	1.08E-03	T	0.43	0.05		6.66E-04	T	0.46	0.06	0.88293
41685_0	6	20,506,152	A	G	0.90	1.66E-03	G	1.53	0.05		2.29E-05	G	2.41	0.13	0.94925
41691_36	6	20,549,938	A	G	0.90	1.66E-03	G	1.53	0.05		2.29E-05	G	2.41	0.13	0.94925
43146_121	6	35,503,563	G	C	0.80	1.13E-04	C	0.79	0.07		5.01E-05	C	0.87	0.10	0.89657
43147_81	6	35,503,563	G	C	0.80	1.13E-04	C	0.79	0.07		5.01E-05	C	0.87	0.10	0.89657
44286_48	6	45,342,882	C	T	0.86	1.89E-05	C	0.70	0.09		6.49E-05	C	0.62	0.07	0.89906
52187_41	5	3,612,553	T	C	0.80	2.41E-04	C	0.75	0.06		9.93E-04	C	0.73	0.06	0.92623

22850_134	2	35,442,434	G	T	0.69					6.69E-05	G	0.69	0.14	1.32E-04	G	0.64	0.12	0.84482
22852_76	2	35,442,434	G	T	0.69					6.69E-05	G	0.69	0.14	1.32E-04	G	0.64	0.12	0.84482
22857_54	2	35,444,258	A	T	0.69					1.39E-03	T	0.58	0.07	1.51E-05	T	1.28	0.40	0.76255
24710_141	2	54,179,913	T	G	0.83					1.60E-04	G	0.59	0.14	4.59E-05	T	0.75	0.15	0.83518
35965_139	4	7,461,542	A	T	0.89					1.09E-03	T	2.09	0.08	2.82E-06	T	4.55	0.39	0.92152
36035_144	4	7,529,193	C	T	0.89					1.09E-03	T	2.09	0.08	2.82E-06	T	4.55	0.39	0.92152
42188_0	6	26,519,439	A	T	0.57					1.57E-03	A	0.38	0.05	1.29E-04	A	0.61	0.11	0.84306
43139_147	6	35,410,749	T	A	0.94					3.24E-06	A	2.32	0.21	1.21E-03	A	1.29	0.08	0.94321
44004_89	6	43,055,851	A	T	0.56					7.81E-04	T	0.45	0.04	3.01E-07	T	1.62	0.70	0.69883
44006_90	6	43,055,851	A	T	0.56					7.81E-04	T	0.45	0.04	3.01E-07	T	1.62	0.70	0.69883
18259_138	1	23,320,278	A	T	0.86	2.29E-06	T	1.20	0.10	8.96E-06	T	1.40	0.13	2.21E-04	T	0.92	0.09	0.90827
18261_97	1	23,320,260	C	T	0.86	2.29E-06	T	1.20	0.10	8.96E-06	T	1.40	0.13	2.21E-04	T	0.92	0.09	0.90827
19549_15	1	37,741,966	T	C	0.91	1.76E-04	C	1.00	0.06	1.69E-05	C	1.41	0.12	3.63E-05	C	1.29	0.12	0.91653
21485_0	1	7,307,696	T	G	0.92	3.29E-05	G	0.89	0.08	9.75E-04	G	1.25	0.15	2.19E-04	G	0.71	0.05	0.93803
21848_130	2	14,625,705	T	G	0.63	2.46E-05	G	0.63	0.08	9.98E-04	G	0.59	0.07	1.49E-06	G	1.02	0.21	0.8268
22333_22	2	26,271,297	A	C	0.92	5.16E-04	A	0.63	0.06	1.13E-11	A	2.86	0.76	1.08E-05	A	1.28	0.16	0.89148
22556_81	2	30,009,497	C	G	0.81	1.51E-03	G	2.25	0.06	1.53E-03	G	2.94	0.11	8.09E-04	G	2.70	0.09	0.96798
22556_82	2	30,009,498	T	G	0.81	1.51E-03	G	2.25	0.06	2.05E-04	G	3.89	0.19	2.99E-05	G	3.97	0.22	0.94661
28920_49	3	51,747,286	G	C	0.76	1.65E-05	C	0.86	0.08	5.25E-04	C	0.72	0.06	3.68E-08	C	2.05	0.47	0.81437
34423_135	4	38,572,767	G	T	0.63	5.80E-04	G	0.54	0.05	2.64E-06	G	1.00	0.19	2.77E-04	G	0.62	0.07	0.90012
34444_0	4	38,968,143	A	G	0.63	5.80E-04	A	0.54	0.05	2.64E-06	A	1.00	0.19	2.77E-04	A	0.62	0.07	0.90012
34921_15	4	51,576,446	C	T	0.84	2.04E-07	T	1.45	0.12	2.62E-05	T	1.40	0.10	3.27E-05	T	1.23	0.09	0.92982
35048_13	4	54,315,023	T	C	0.87	8.43E-06	C	1.03	0.09	5.75E-04	C	0.89	0.06	4.78E-09	C	1.82	0.29	0.86031
35059_0	4	54,428,146	T	A	0.87	8.43E-06	A	1.03	0.09	5.75E-04	A	0.89	0.06	4.78E-09	A	1.82	0.29	0.86031
35574_65	4	65,313,898	A	G	0.53	1.32E-04	G	0.49	0.07	1.23E-04	G	0.95	0.29	2.81E-04	G	0.53	0.07	0.878
35889_99	4	73,207,403	G	C	0.77	8.36E-06	C	0.92	0.09	1.45E-06	C	1.41	0.18	1.34E-05	C	1.08	0.11	0.90934
35901_47	4	73,657,465	C	T	0.77	8.36E-06	T	0.92	0.09	2.84E-04	T	0.84	0.07	1.33E-05	T	0.90	0.08	0.91501

35902_71	4	73,657,613	T	C	0.77	3.90E-06	C	0.95	0.10	1.40E-04	C	0.91	0.07	5.34E-06	C	1.08	0.11	0.9076
35911_0	4	73,804,913	T	C	0.77	3.90E-06	C	0.95	0.10	1.40E-04	C	0.91	0.07	5.34E-06	C	1.08	0.11	0.9076
37554_0	5	1,060,000	T	A	0.59	3.47E-05	T	0.41	0.08	4.48E-04	T	0.42	0.06	1.40E-04	T	0.40	0.09	0.81844
37554_100	5	1,060,049	C	G	0.59	3.47E-05	C	0.41	0.08	9.56E-05	C	0.51	0.09	7.95E-10	C	1.33	0.87	0.60624
37655_144	5	1,145,178	G	T	0.60	2.36E-04	G	0.42	0.07	2.49E-04	G	0.47	0.09	6.92E-07	G	1.18	0.39	0.75232
37656_16	5	1,145,021	A	T	0.60	2.36E-04	A	0.42	0.07	2.49E-04	A	0.47	0.09	6.92E-07	A	1.18	0.39	0.75232
43146_145	6	35,503,587	C	G	0.80	4.98E-04	G	0.64	0.06	3.96E-04	G	0.73	0.07	2.85E-04	C	0.48	0.06	0.89446
43147_57	6	35,503,587	C	G	0.80	4.98E-04	G	0.64	0.06	3.96E-04	G	0.73	0.07	2.85E-04	C	0.48	0.06	0.89446
44114_148	6	43,778,047	T	A	0.83	2.75E-05	A	0.93	0.08	1.42E-04	A	0.92	0.08	4.08E-04	A	0.70	0.13	0.84598
44114_149	6	43,778,046	G	A	0.83	2.75E-05	A	0.93	0.08	1.42E-04	A	0.92	0.08	4.08E-04	A	0.70	0.13	0.84598

Supplemental table 3. Genomic context of ascorbic acid significantly associated anchored genes

Model	Marker	Chromosome	Position	Gene ID	Function
GLM	18446_91	1	26,086,756	Spo11186.1	Zinc finger CCCH domain-containing protein 29
GLM	18447_135	1	26,224,404	Spo11187.1	Double Clp-N motif-containing P-loop nucle
GLM	18997_127	1	32,069,169	Spo18501.1	Ankyrin repeat family protein
GLM	19022_139	1	32,206,058	Spo18441.1	S-adenosyl-methyltransferase mraw, putative
GLM	19027_0	1	32,243,743	Spo18492.1	DNA (cytosine-5)-methyltransferase (2.1.1.37)
GLM	19031_147	1	32,253,488	Spo18438.1	(RAP Annotation release2) Galact
GLM	19031_71	1	32,253,412	Spo18438.1	(RAP Annotation release2) Galact
GLM	19032_111	1	32,253,488	Spo18438.1	(RAP Annotation release2) Galact
GLM	19037_84	1	32,307,120	Spo18434.1	Protein arginine N-methyltransferase
GLM	19355_0	1	36,206,961	Spo16086.1	Unknown protein
GLM	19355_71	1	36,207,039	Spo16086.1	Unknown protein
GLM	19513_51	1	37,511,412	Spo25658.1	Methyltransferase-like
GLM	19514_17	1	37,511,842	Spo25658.1	Methyltransferase-like
GLM	19819_142	1	39,795,591	Spo10570.1	Zinc ion binding protein
GLM	19832_42	1	39,863,270	Spo10599.1	40S ribosomal protein S13, putative
GLM	19968_37	1	40,913,370	Spo10738.1	Equilibrative nucleoside transporter 6
GLM	19968_80	1	40,913,413	Spo10738.1	Equilibrative nucleoside transporter 6
GLM	20013_59	1	41,230,635	Spo10761.1	Pectin lyase-like protein
GLM	20014_86	1	41,230,635	Spo10761.1	Pectin lyase-like protein
GLM	20248_143	1	43,534,058	Spo07624.1	(Cytochrome P450, putative) (1.14.13.68)
GLM	20248_143	1	43,534,058	Spo07597.1	Zinc finger family protein

GLM	20250_0	1	43,534,176	Spo07624.1	(Cytochrome P450, putative) (1.14.13.68)
GLM	20250_0	1	43,534,176	Spo07597.1	Zinc finger family protein
GLM	21099_124	1	49,342,208	Spo05862.1	Pentatricopeptide repeat-containing protein, putative
GLM	21099_147	1	49,342,231	Spo05862.1	Pentatricopeptide repeat-containing protein, putative
GLM	21115_131	1	49,380,455	Spo05860.1	RING/U-box superfamily protein
GLM	21115_133	1	49,380,453	Spo05860.1	RING/U-box superfamily protein
GLM	21255_99	1	50,390,815	Spo25332.1	Ribose-phosphate pyrophosphokinase 4 (2.7.6.1) (Phosphoribosyl pyrophosphate synthase 4)
GLM	21256_74	1	50,390,815	Spo25332.1	Ribose-phosphate pyrophosphokinase 4 (2.7.6.1) (Phosphoribosyl pyrophosphate synthase 4)
GLM	21292_120	1	5,140,768	Spo26073.1	RS2-interacting KH protein, putative
GLM	21292_132	1	5,140,780	Spo26073.1	RS2-interacting KH protein, putative
GLM	21292_98	1	5,140,746	Spo26073.1	RS2-interacting KH protein, putative
GLM	21294_108	1	5,140,822	Spo26073.1	RS2-interacting KH protein, putative
GLM	21472_119	1	7,236,734	Spo17760.1	Soluble inorganic pyrophosphatase
GLM	21473_74	1	7,236,734	Spo17760.1	Soluble inorganic pyrophosphatase
GLM	21500_21	1	7,433,915	Spo17780.1	DNA helicase (3.6.4.12)
GLM	21513_40	1	7,907,820	Spo00901.1	Peroxidase (1.11.1.7)
GLM	21522_144	1	7,976,203	Spo00903.1	chromatin remodeling 4
GLM	22329_69	2	25,391,528	Spo26753.1	Unknown protein
GLM	22449_54	2	28,231,032	Spo15805.1	Like-COV protein
GLM	22452_66	2	28,293,382	Spo15806.1	DUF4378 domain protein

GLM	22571_88	2	30,189,324	Spo15561.1	YABBY-like transcription factor PROLONGATA
GLM	22576_7	2	30,257,405	Spo15553.1	Nucleoporin-like protein
GLM	22578_49	2	30,280,193	Spo15559.1	Equilibrative nucleoside transporter family protein
GLM	22594_0	2	30,388,094	Spo15557.1	serine/threonine protein kinase 1
GLM	22884_92	2	35,733,419	Spo14828.1	IQ-domain 1
GLM	22884_98	2	35,733,425	Spo14828.1	IQ-domain 1
GLM	22885_109	2	35,733,738	Spo14828.1	IQ-domain 1
GLM	22885_111	2	35,733,740	Spo14828.1	IQ-domain 1
GLM	23191_35	2	40,173,581	Spo14444.1	ARC6
GLM	23195_145	2	40,183,489	Spo14464.1	Calmodulin (CaM)
GLM	23199_95	2	40,231,531	Spo14447.1	(CCHC-type zinc knuckle protein) (DNA-binding protein)
GLM	23213_66	2	40,459,411	Spo14471.1	Adaptor protein complex 3 subunit sigma
GLM	23225_86	2	40,605,455	Spo05017.1	Unknown protein
GLM	23233_143	2	40,671,312	Spo05020.1	Plant protein of unknown function (DUF639)
GLM	23424_75	2	42,700,972	Spo02824.1	Eukaryotic aspartyl protease family protein
GLM	23431_96	2	42,735,301	Spo02836.1	Protein glycosylationmyb-like TTH transcriptional regulator
GLM	23432_145	2	42,747,240	Spo02837.1	Golgi transport complex family protein
GLM	23448_95	2	42,872,793	Spo02828.1	Dead box ATP-dependent RNA helicase, putative
GLM	23601_0	2	4,504,336	Spo19126.1	Transforming growth factor-beta receptor-associated protein 1 isoform 1

GLM	23637_44	2	4,549,925	Spo19124.1	Lysine ketoglutarate reductase trans-splicing protein
GLM	24058_64	2	49,652,698	Spo01559.1	Arabidopsis thaliana protein of unknown function (DUF821)
GLM	24058_66	2	49,652,696	Spo01559.1	Arabidopsis thaliana protein of unknown function (DUF821)
GLM	24704_131	2	54,129,556	Spo23855.1	F-box family protein
GLM	24704_77	2	54,129,502	Spo23855.1	F-box family protein
GLM	24705_147	2	54,129,556	Spo23855.1	F-box family protein
GLM	24962_121	2	55,727,156	Spo28008.1	RING/U-box superfamily protein
GLM	24963_109	2	55,727,077	Spo28008.1	RING/U-box superfamily protein
GLM	25316_140	2	58,214,345	Spo01212.1	Transducin family protein/WD-40 repeat protein
GLM	25316_144	2	58,214,349	Spo01212.1	Transducin family protein/WD-40 repeat protein
GLM	25648_103	2	60,396,323	Spo19502.1	Alpha/beta-Hydrolases superfamily protein
GLM	25650_46	2	60,431,729	Spo19489.1	Ca2 :Cation Antiporter (CaCA) Family putative
GLM	26394_140	3	11,201,518	Spo08335.1	Fip1 [V]-like protein
GLM	26394_71	3	11,201,449	Spo08335.1	Fip1 [V]-like protein
GLM	27513_96	3	2,579,672	Spo04375.1	Calcium-dependent lipid-binding domain-containing protein
GLM	27514_54	3	2,579,672	Spo04375.1	Calcium-dependent lipid-binding domain-containing protein
GLM	27590_0	3	2,689,687	Spo04443.1	Disease resistance protein
GLM	27687_0	3	2,808,515	Spo04394.1	MADS-box transcription factor 21

GLM	27688_32	3	2,808,921	Spo04394.1	MADS-box transcription factor 21
GLM	27858_148	3	3,148,769	Spo04468.1	Kinase family protein
GLM	27964_22	3	3,406,045	Spo04483.1	RNA polymerase III subunit-like protein
GLM	28020_33	3	3,540,253	Spo04429.1	Exocyst subunit exo70 family protein G1
GLM	28077_143	3	3,688,025	Spo15965.1	Leucine-rich receptor-like kinase family protein
GLM	28108_149	3	3,743,058	Spo16012.1	P-loop containing nucleoside triphosphate hydrolases superfamily protein
GLM	28117_142	3	3,745,218	Spo16012.1	P-loop containing nucleoside triphosphate hydrolases superfamily protein
GLM	28177_11	3	3,853,344	Spo15970.1	Transducin/WD40 repeat-like superfamily protein
GLM	28185_72	3	3,870,900	Spo15971.1	Leucine-rich receptor-like kinase family protein, putative
GLM	28250_142	3	3,943,167	Spo16018.1	(Acidic chitinase) (Acidic endochitinase)
GLM	28250_75	3	3,943,100	Spo16018.1	(Acidic chitinase) (Acidic endochitinase)
GLM	28263_78	3	3,956,975	Spo16021.1	Pentatricopeptide repeat-containing protein, putative
GLM	28263_92	3	3,956,961	Spo16021.1	Pentatricopeptide repeat-containing protein, putative
GLM	28549_88	3	4,427,955	Spo11326.1	5' nucleotidase family protein
GLM	28550_27	3	4,428,115	Spo16044.1	Protein FAR1-RELATED SEQUENCE 5
GLM	28937_41	3	51,955,976	Spo21502.1	Vacuolar protein sorting-associated protein 13C, putative
GLM	28939_73	3	51,962,072	Spo21502.1	Vacuolar protein sorting-associated protein 13C, putative

GLM	28939_75	3	51,962,074	Spo21502.1	Vacuolar protein sorting-associated protein 13C, putative
GLM	28947_0	3	51,994,924	Spo21505.1	Tetratricopeptide repeat (TPR)-like superfamily protein
GLM	29531_45	3	6,270,145	Spo02600.1	Histone-lysine N-methyltransferase
GLM	29532_136	3	6,270,145	Spo02600.1	Histone-lysine N-methyltransferase
GLM	29555_0	3	6,322,399	Spo02521.1	GTP-binding protein
GLM	29557_0	3	6,334,215	Spo02604.1	Cytochrome P450 family 71 protein
GLM	29573_53	3	6,361,232	Spo28031.1	NBS-LRR disease resistance protein-like
GLM	29583_0	3	6,386,626	Spo02526.1	Oligopeptide transporter 6
GLM	29583_92	3	6,386,683	Spo02526.1	Oligopeptide transporter 6
GLM	29649_30	3	6,475,814	Spo02614.1	NBS-LRR disease resistance protein
GLM	29706_149	3	655,179	Spo12735.1	E3 ubiquitin protein ligase RIN3 (6.3.2.-) (RPM1-interacting protein 3)
GLM	29947_8	3	699,742	Spo12735.1	E3 ubiquitin protein ligase RIN3 (6.3.2.-) (RPM1-interacting protein 3)
GLM	30158_28	3	74,370,145	Spo25294.1	Fcf2 pre-rRNA processing protein
GLM	30163_65	3	74,503,916	Spo15225.1	Unknown protein
GLM	32481_143	4	115,303,744	Spo09123.1	Serine/threonine-protein kinase (2.7.11.1)
GLM	32519_92	4	115,809,492	Spo26522.1	Dentin sialophosphoprotein-related, putative isoform 1
GLM	32520_56	4	115,809,492	Spo26522.1	Dentin sialophosphoprotein-related, putative isoform 1
GLM	33681_148	4	18,084,042	Spo24677.1	Ammonium transporter
GLM	33683_0	4	18,108,994	Spo24677.1	Ammonium transporter
GLM	33757_0	4	19,145,345	Spo12100.1	Ankyrin repeat-containing protein
GLM	33757_12	4	19,145,482	Spo12100.1	Ankyrin repeat-containing protein

GLM	34733_43	4	47,437,830	Spo06713.1	Anaphase-promoting complex subunit 5
GLM	34738_120	4	47,544,863	Spo20475.1	DNA-directed RNA polymerase (2.7.7.6)
GLM	34883_38	4	51,195,861	Spo11659.1	tRNA (guanine(37)-N1)-methyltransferase (2.1.1.228) (M1G-methyltransferase) (tRNA [GM37] methyltransferase) (tRNA methyltransferase 5 homolog)
GLM	34901_12	4	51,448,355	Spo11659.1	tRNA (guanine(37)-N1)-methyltransferase (2.1.1.228) (M1G-methyltransferase) (tRNA [GM37] methyltransferase) (tRNA methyltransferase 5 homolog)
GLM	34913_24	4	51,557,723	Spo25192.1	Single-stranded nucleic acid-binding protein R3H
GLM	34989_118	4	5,333,134	Spo15071.1	Tubulin alpha chain, putative
GLM	34990_9	4	5,337,392	Spo15092.1	Ethylene-responsive transcription factor
GLM	34990_9	4	5,337,392	Spo15072.1	Unknown protein
GLM	35087_0	4	55,154,629	Spo24720.1	(BnaC09g16480D protein) (BnaC09g26880D protein) (BnaC09g29120D protein) (BnaUnng00820D protein)
GLM	35093_1	4	55,155,496	Spo24720.1	(BnaC09g16480D protein) (BnaC09g26880D protein) (BnaC09g29120D protein) (BnaUnng00820D protein)
GLM	35965_128	4	7,461,531	Spo11988.1	Saccharopine dehydrogenase
GLM	35966_107	4	7,461,531	Spo11988.1	Saccharopine dehydrogenase
GLM	36247_148	4	78,781,464	Spo17004.1	PAP-specific phosphatase

GLM	36249_0	4	78,799,506	Spo17004.1	PAP-specific phosphatase
GLM	36675_142	4	85,449,268	Spo14288.1	Unknown protein
GLM	36678_0	4	85,593,100	Spo13257.1	Unknown protein
GLM	39454_145	5	37,592,017	Spo16492.1	Early-responsive to dehydration stress family protein
GLM	39454_146	5	37,592,018	Spo16492.1	Early-responsive to dehydration stress family protein
GLM	39469_0	5	37,826,724	Spo16496.1	Protein BREAST CANCER SUSCEPTIBILITY 1-like protein
GLM	39491_1	5	39,065,637	Spo22622.1	Multidrug resistance protein ABC transporter family protein
GLM	39497_65	5	39,158,874	Spo22622.1	Multidrug resistance protein ABC transporter family protein
GLM	39497_8	5	39,158,817	Spo22622.1	Multidrug resistance protein ABC transporter family protein
GLM	39497_93	5	39,158,902	Spo22622.1	Multidrug resistance protein ABC transporter family protein
GLM	39553_111	5	40,016,036	Spo15859.1	Gamete expressed 2
GLM	39553_133	5	40,016,058	Spo15859.1	Gamete expressed 2
GLM	39553_80	5	40,016,005	Spo15859.1	Gamete expressed 2
GLM	39554_44	5	40,016,218	Spo15859.1	Gamete expressed 2
GLM	39973_67	5	47,389,521	Spo12601.1	Glutathione reductase
GLM	39977_7	5	47,393,417	Spo12601.1	Glutathione reductase
GLM	40570_125	5	64,568,044	Spo06630.1	Transport Sec24 family protein
GLM	40570_132	5	64,568,051	Spo06630.1	Transport Sec24 family protein
GLM	41044_24	5	9,280,921	Spo24381.1	Nitrate transporter NRT1.2B
GLM	41044_26	5	9,280,919	Spo24381.1	Nitrate transporter NRT1.2B

GLM	41320_124	6	14,602,008	Spo09215.1	Phosphatase 2C family protein
GLM	41320_140	6	14,602,024	Spo09215.1	Phosphatase 2C family protein
GLM	41588_88	6	18,864,209	Spo06041.1	Serine/arginine repetitive matrix protein 2 isoform 2
GLM	41596_81	6	18,974,282	Spo05526.1	Unknown protein
GLM	41833_30	6	21,987,025	Spo17542.1	Gb|AAF01580.1
GLM	42736_61	6	32,402,383	Spo26944.1	Cytochrome P450, putative
GLM	42739_0	6	32,403,159	Spo26944.1	Cytochrome P450, putative
GLM	43614_13	6	39,692,322	Spo11599.1	DCD (Development and cell death) domain protein
GLM	43614_25	6	39,692,334	Spo11599.1	DCD (Development and cell death) domain protein
GLM	44214_102	6	44,765,221	Spo23235.1	Vacuolar protein sorting-associated protein 25 (AtVPS25) (ESCRT-II complex subunit VPS25)
GLM	44214_141	6	44,765,260	Spo23235.1	Vacuolar protein sorting-associated protein 25 (AtVPS25) (ESCRT-II complex subunit VPS25)
GLM	44273_61	6	45,310,152	Spo11396.1	Receptor-like kinase
GLM	44277_51	6	45,336,145	Spo11367.1	Amine oxidase
GLM	44286_49	6	45,342,881	Spo11398.1	(Cytochrome P450, putative) (1.14.13.88)
GLM	44286_64	6	45,342,866	Spo11398.1	(Cytochrome P450, putative) (1.14.13.88)
GLM, P3D	20279_125	1	43,647,136	Spo07603.1	Pyridoxamine 5'-phosphate oxidase family protein
GLM, P3D	20279_78	1	43,647,089	Spo07603.1	Pyridoxamine 5'-phosphate oxidase family protein

GLM, P3D	20965_149	1	48,593,828	Spo05899.1	oxidoreductases, acting on NADH or NADPH
GLM, P3D	20970_7	1	48,610,543	Spo06001.1	Inositol-tetrakisphosphate 1-kinase (2.7.1.134)
GLM, P3D	21101_74	1	49,342,286	Spo05862.1	Pentatricopeptide repeat-containing protein, putative
GLM, P3D	21113_147	1	49,380,453	Spo05860.1	RING/U-box superfamily protein
GLM, P3D	21250_98	1	50,330,048	Spo05903.1	F-box family protein
GLM, P3D	21252_144	1	50,330,048	Spo05903.1	F-box family protein
GLM, P3D	21464_139	1	7,152,451	Spo17786.1	Transmembrane protein, putative
GLM, P3D	21464_77	1	7,152,389	Spo17786.1	Transmembrane protein, putative
GLM, P3D	21465_15	1	7,152,451	Spo17786.1	Transmembrane protein, putative
GLM, P3D	21465_77	1	7,152,389	Spo17786.1	Transmembrane protein, putative
GLM, P3D	21854_52	2	14,931,082	Spo13279.1	30S ribosomal protein S7, chloroplastic
GLM, P3D	22327_141	2	25,043,877	Spo26758.1	Exostosin family protein
GLM, P3D	22327_145	2	25,043,873	Spo26758.1	Exostosin family protein
GLM, P3D	23442_135	2	42,838,538	Spo02828.1	Dead box ATP-dependent RNA helicase, putative
GLM, P3D	23446_72	2	42,872,697	Spo02828.1	Dead box ATP-dependent RNA helicase, putative
GLM, P3D	24704_113	2	54,129,538	Spo23855.1	F-box family protein
GLM, P3D	27548_30	3	2,604,203	Spo04438.1	Myb transcription factor
GLM, P3D	27616_18	3	2,712,980	Spo04385.1	CC-NBS-LRR disease resistance protein
GLM, P3D	27657_47	3	2,759,377	Spo04391.1	Beta-carotene hydroxylase
GLM, P3D	27876_41	3	3,192,000	Spo04416.1	ATP binding protein, putative
GLM, P3D	27964_0	3	3,406,023	Spo04483.1	RNA polymerase III subunit-like protein
GLM, P3D	28308_146	3	40,243,417	Spo11326.1	5' nucleotidase family protein

GLM, P3D	28906_0	3	51,645,416	Spo20642.1	Unknown protein
GLM, P3D	31356_46	3	97,879,214	Spo18669.1	Casparian strip membrane protein 2 (TpCASP2)
GLM, P3D	31357_99	3	97,879,214	Spo18669.1	Casparian strip membrane protein 2 (TpCASP2)
GLM, P3D	32486_135	4	115,313,459	Spo28079.1	Serine/threonine-protein kinase (2.7.11.1)
GLM, P3D	32487_78	4	115,313,459	Spo28079.1	Serine/threonine-protein kinase (2.7.11.1)
GLM, P3D	34907_53	4	51,498,162	Spo00967.1	Endoribonuclease L-PSP family protein
GLM, P3D	34921_27	4	51,576,458	Spo25192.1	Single-stranded nucleic acid-binding protein R3H
GLM, P3D	35053_25	4	5,432,524	Spo15083.1	Acyl-activating enzyme 14
GLM, P3D	35127_141	4	5,581,079	Spo00676.1	(BnaAnng17230D protein) (BnaAnng37090D protein)
GLM, P3D	35926_33	4	74,211,414	Spo15279.1	BnaC05g24240D protein
GLM, P3D	35926_79	4	74,211,460	Spo15279.1	BnaC05g24240D protein
GLM, P3D	40217_111	5	5,266,118	Spo00293.1	Unknown protein
GLM, P3D	40217_119	5	5,266,126	Spo00293.1	Unknown protein
GLM, P3D	40599_0	5	65,931,815	Spo05408.1	Ubiquitin family protein
GLM, P3D	40651_37	5	67,866,717	Spo18549.1	Translocation protein SEC63-like protein
GLM, P3D	41833_33	6	21,987,022	Spo17542.1	Gb|AAF01580.1
GLM, P3D	42716_59	6	32,370,727	Spo26891.1	Superoxide dismutase [Cu-Zn] (1.15.1.1)
GLM, P3D	42718_109	6	32,370,727	Spo26891.1	Superoxide dismutase [Cu-Zn] (1.15.1.1)
GLM, P3D	44281_23	6	45,342,253	Spo11398.1	(Cytochrome P450, putative) (1.14.13.88)
GLM, P3D, perMarker	18259_138	1	23,320,278	Spo25359.1	Unknown protein
GLM, P3D, perMarker	18261_97	1	23,320,260	Spo25359.1	Unknown protein

GLM, P3D, perMarker	19549_15	1	37,741,966	Spo25607.1	Receptor-like kinase
GLM, P3D, perMarker	21485_0	1	7,307,696	Spo17757.1	Sec23/Sec24 protein transport family protein
GLM, P3D, perMarker	21848_130	2	14,625,705	Spo13276.1	SAUR-like auxin-responsive family protein
GLM, P3D, perMarker	22333_22	2	26,271,297	Spo28197.1	Anti-microbial protein 2
GLM, P3D, perMarker	22556_81	2	30,009,497	Spo01408.1	Glucan endo-1,3-beta-glucosidase 11 (3.2.1.39) ((1->3)-beta-glucan endohydrolase 11) ((1->3)-beta-glucanase 11) (Beta-1,3-endoglucanase 11) (Beta-1,3-glucanase 11) (Precursor)
GLM, P3D, perMarker	22556_82	2	30,009,498	Spo01408.1	Glucan endo-1,3-beta-glucosidase 11 (3.2.1.39) ((1->3)-beta-glucan endohydrolase 11) ((1->3)-beta-glucanase 11) (Beta-1,3-endoglucanase 11) (Beta-1,3-glucanase 11) (Precursor)
GLM, P3D, perMarker	28920_49	3	51,747,286	Spo20633.1	Unknown protein
GLM, P3D, perMarker	34423_135	4	38,572,767	Spo24573.1	Peptide-N4-(N-acetyl-beta-glucosaminyl)asparagine amidase A protein
GLM, P3D, perMarker	34444_0	4	38,968,143	Spo24572.1	Salt overly sensitive 1
GLM, P3D, perMarker	34921_15	4	51,576,446	Spo25192.1	Single-stranded nucleic acid-binding protein R3H

GLM, P3D, perMarker	35048_13	4	54,315,023	Spo03750.1	Ulp1 protease family, carboxy-terminal domain protein
GLM, P3D, perMarker	35059_0	4	54,428,146	Spo19617.1	F23A5.27 isoform 5
GLM, P3D, perMarker	35574_65	4	65,313,898	Spo09910.1	Unknown protein
GLM, P3D, perMarker	35889_99	4	73,207,403	Spo15272.1	Aldehyde dehydrogenase family 2 member B4
GLM, P3D, perMarker	35901_47	4	73,657,465	Spo15274.1	Tir-nbs-lrr resistance protein, putative isoform 3
GLM, P3D, perMarker	35902_71	4	73,657,613	Spo15274.1	Tir-nbs-lrr resistance protein, putative isoform 3
GLM, P3D, perMarker	35911_0	4	73,804,913	Spo15276.1	Protease Do-like 9
GLM, P3D, perMarker	37554_0	5	1,060,000	Spo17628.1	Beta-xylosidase 2
GLM, P3D, perMarker	37554_100	5	1,060,049	Spo17628.1	Beta-xylosidase 2
GLM, P3D, perMarker	37655_144	5	1,145,178	Spo17718.1	BRCT domain-containing protein
GLM, P3D, perMarker	37656_16	5	1,145,021	Spo17718.1	BRCT domain-containing protein
GLM, P3D, perMarker	43146_145	6	35,503,587	Spo09335.1	2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase (4.6.1.12)
GLM, P3D, perMarker	43147_57	6	35,503,587	Spo09335.1	2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase (4.6.1.12)
GLM, P3D, perMarker	44114_148	6	43,778,047	Spo23394.1	M20 peptidase acetylornithine deacetylase

GLM, P3D, perMarker	44114_149	6	43,778,046	Spo23394.1	M20 peptidase acetylornithine deacetylase
GLM, perMarker	19026_0	1	32,220,045	Spo18440.1	Lysine-specific histone demethylase 1 homolog 1 (1.-.-.-) (Flavin-containing amine oxidase domain-containing protein 1) (Protein LSD1-LIKE 1)
GLM, perMarker	19553_50	1	37,742,824	Spo25607.1	Receptor-like kinase
GLM, perMarker	19968_75	1	40,913,408	Spo10738.1	Equilibrative nucleoside transporter 6
GLM, perMarker	19969_15	1	40,913,321	Spo10738.1	Equilibrative nucleoside transporter 6
GLM, perMarker	19970_45	1	40,913,490	Spo10738.1	Equilibrative nucleoside transporter 6
GLM, perMarker	19970_58	1	40,913,477	Spo10738.1	Equilibrative nucleoside transporter 6
GLM, perMarker	23185_98	2	40,083,391	Spo14440.1	Lysine-specific histone demethylase 1 homolog 3 (1.-.-.-) (Flavin-containing amine oxidase domain-containing protein 3) (Protein LSD1-like 3)
GLM, perMarker	23187_99	2	40,083,391	Spo14440.1	Lysine-specific histone demethylase 1 homolog 3 (1.-.-.-) (Flavin-containing amine oxidase domain-containing protein 3) (Protein LSD1-like 3)
GLM, perMarker	23196_117	2	40,183,547	Spo14464.1	Calmodulin (CaM)
GLM, perMarker	23196_60	2	40,183,604	Spo14464.1	Calmodulin (CaM)
GLM, perMarker	23430_93	2	42,735,301	Spo02836.1	Protein glycosylationmyb-like TTH transcriptional regulator
GLM, perMarker	23453_18	2	44,088,756	Spo20595.1	CHY zinc finger family protein, expressed
GLM, perMarker	27890_4	3	3,255,946	Spo04476.1	Chloroplast thylakoid membrane, putative isoform 1

GLM, perMarker	27964_27	3	3,406,050	Spo04483.1	RNA polymerase III subunit-like protein
GLM, perMarker	28266_112	3	3,962,960	Spo16023.1	LIM domain-containing protein A, putative isoform 1
GLM, perMarker	28266_79	3	3,962,927	Spo16023.1	LIM domain-containing protein A, putative isoform 1
GLM, perMarker	28280_119	3	4,003,122	Spo16025.1	Multidrug resistance protein ABC transporter family
GLM, perMarker	28280_144	3	4,003,097	Spo16025.1	Multidrug resistance protein ABC transporter family
GLM, perMarker	28308_0	3	40,243,271	Spo11326.1	5' nucleotidase family protein
GLM, perMarker	31361_95	3	97,879,861	Spo18669.1	Casparian strip membrane protein 2 (TpCASP2)
GLM, perMarker	31364_144	3	97,914,322	Spo18659.1	Pentatricopeptide repeat-containing protein
GLM, perMarker	32481_142	4	115,303,743	Spo09123.1	Serine/threonine-protein kinase (2.7.11.1)
GLM, perMarker	33279_54	4	122,232,961	Spo18177.1	Unknown protein
GLM, perMarker	33294_0	4	122,384,809	Spo18189.1	receptor kinase 2
GLM, perMarker	34683_81	4	46,896,647	Spo00594.1	Unknown protein
GLM, perMarker	34683_82	4	46,896,646	Spo00594.1	Unknown protein
GLM, perMarker	34999_144	4	53,489,507	Spo22693.1	Guanine nucleotide-exchange, putative
GLM, perMarker	35000_107	4	53,489,476	Spo22693.1	Guanine nucleotide-exchange, putative
GLM, perMarker	35000_76	4	53,489,507	Spo22693.1	Guanine nucleotide-exchange, putative
GLM, perMarker	35023_20	4	53,780,508	Spo23999.1	Oxidoreductases, putative isoform 2
GLM, perMarker	35573_123	4	65,313,687	Spo09910.1	Unknown protein
GLM, perMarker	35912_34	4	73,805,544	Spo15276.1	Protease Do-like 9
GLM, perMarker	35913_112	4	73,805,544	Spo15276.1	Protease Do-like 9
GLM, perMarker	39285_20	5	3,359,022	Spo02353.1	Serine/arginine-rich splicing factor 4

GLM, perMarker	39553_130	5	40,016,055	Spo15859.1	Gamete expressed 2
GLM, perMarker	40217_108	5	5,266,115	Spo00293.1	Unknown protein
GLM, perMarker	40217_110	5	5,266,117	Spo00293.1	Unknown protein
GLM, perMarker	41685_0	6	20,506,152	Spo03863.1	Pentatricopeptide repeat superfamily protein
GLM, perMarker	41691_36	6	20,549,938	Spo03865.1	Photosystem II CP47 chlorophyll apoprotein
GLM, perMarker	43146_121	6	35,503,563	Spo09335.1	2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase (4.6.1.12)
GLM, perMarker	43147_81	6	35,503,563	Spo09335.1	2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase (4.6.1.12)
GLM, perMarker	44286_48	6	45,342,882	Spo11398.1	(Cytochrome P450, putative) (1.14.13.88)
GLM, perMarker	52187_41	5	3,612,553	Spo02244.1	Protease inhibitor/seed storage/lipid transfer family protein
P3D	17631_35	1	10,773,565	Spo27163.1	50S ribosomal protein L1
P3D	17830_19	1	13,647,788	Spo11862.1	IMP dehydrogenase/GMP reductase, putative
P3D	17835_68	1	13,661,581	Spo11863.1	Pollen Ole e 1 allergen and extensin family protein
P3D	18420_84	1	25,954,646	Spo11159.1	Anaphase-promoting complex subunit 1
P3D	18421_149	1	25,954,646	Spo11159.1	Anaphase-promoting complex subunit 1
P3D	18773_51	1	30,188,430	Spo24939.1	Nucleolar complex protein 2 isoform 1
P3D	18779_148	1	30,323,142	Spo28215.1	(Kinase, putative) (2.7.11.25)
P3D	18787_147	1	30,343,301	Spo03003.1	E3 SUMO-protein ligase SIZ1
P3D	18813_145	1	30,546,188	Spo02982.1	Albugo candida WGS project CAIX00000000 data, strain Ac Nc2,

					contig AcNc2_CONTIG_5_length_245754
P3D	18813_17	1	30,546,060	Spo02982.1	Albugo candida WGS project CAIX00000000 data, strain Ac Nc2, contig AcNc2_CONTIG_5_length_245754
P3D	18814_24	1	30,546,394	Spo02982.1	Albugo candida WGS project CAIX00000000 data, strain Ac Nc2, contig AcNc2_CONTIG_5_length_245754
P3D	19221_145	1	33,980,168	Spo19424.1	Uro-adherence factor A, putative
P3D	19230_4	1	34,020,770	Spo19454.1	OSBP(Oxysterol-binding protein)-related protein 1C
P3D	19263_96	1	34,763,766	Spo06085.1	Probable magnesium transporter NIPA9
P3D	19263_99	1	34,763,769	Spo06085.1	Probable magnesium transporter NIPA9
P3D	19832_57	1	39,863,285	Spo10599.1	40S ribosomal protein S13, putative
P3D	19837_17	1	39,899,336	Spo11095.1	U-box domain-containing protein 43 (6.3.2.-) (Plant U-box protein 43)
P3D	20338_66	1	44,174,962	Spo09921.1	Quinone oxidoreductase
P3D	21169_65	1	49,642,137	Spo05937.1	OSBP(Oxysterol-binding protein)-related protein 1C
P3D	21170_0	1	49,645,084	Spo05937.1	OSBP(Oxysterol-binding protein)-related protein 1C
P3D	21837_17	2	14,133,093	Spo01010.1	Transmembrane protein, putative
P3D	21837_20	2	14,133,090	Spo01010.1	Transmembrane protein, putative
P3D	21892_32	2	16,614,443	Spo00244.1	Phospholipase A2 family protein

P3D	22554_113	2	29,993,544	Spo01407.1	Early nodulin-16 (N-16) (Precursor)
P3D	22702_0	2	31,412,307	Spo08590.1	Ubiquitin family protein
P3D	23824_53	2	47,802,396	Spo18859.1	Glutathione S-transferase Z1
P3D	23840_113	2	47,917,645	Spo18863.1	Microtubule-associated protein-like
P3D	25069_41	2	56,471,027	Spo21670.1	Amino acid transporter, putative
P3D	25069_62	2	56,471,048	Spo21670.1	Amino acid transporter, putative
P3D	25430_32	2	58,860,729	Spo01178.1	(Receptor protein kinase, putative) (1.3.1.74) (2.7.11.25)
P3D	25431_111	2	58,860,940	Spo01178.1	(Receptor protein kinase, putative) (1.3.1.74) (2.7.11.25)
P3D	25431_117	2	58,860,934	Spo01178.1	(Receptor protein kinase, putative) (1.3.1.74) (2.7.11.25)
P3D	25431_148	2	58,860,903	Spo01178.1	(Receptor protein kinase, putative) (1.3.1.74) (2.7.11.25)
P3D	26255_18	3	108,266,751	Spo11105.1	Unknown protein
P3D	26385_71	3	111,974,439	Spo20855.1	phosphate deficiency response 2
P3D	26565_46	3	12,403,753	Spo14364.1	Small nuclear ribonucleoprotein F (snRNP-F) (Sm protein F)
P3D	26566_147	3	12,403,753	Spo14364.1	Small nuclear ribonucleoprotein F (snRNP-F) (Sm protein F)
P3D	26876_1	3	16,064,491	Spo10239.1	Sugar transporter family protein
P3D	26884_0	3	16,105,912	Spo10220.1	MATE efflux family protein
P3D	28237_70	3	39,199,668	Spo20779.1	S-adenosyl-L-methionine-dependent methyltransferases superfamily protein
P3D	29430_102	3	60,678,645	Spo00044.1	DEAD/DEAH box RNA helicase family protein

P3D	29481_132	3	61,755,981	Spo18603.1	Ras-related small gtp-binding family protein
P3D	29526_47	3	62,474,252	Spo05143.1	S-acyltransferase (2.3.1.225) (Palmitoyltransferase)
P3D	29529_92	3	62,540,090	Spo05146.1	Hydroxyproline-rich glycoprotein family protein
P3D	30083_39	3	72,559,560	Spo01282.1	IQ-domain 6
P3D	30083_6	3	72,559,593	Spo01282.1	IQ-domain 6
P3D	30151_12	3	743,510	Spo12740.1	Invertase
P3D	30156_137	3	743,510	Spo12740.1	Invertase
P3D	30205_109	3	7,568,163	Spo22384.1	Ankyrin repeat family protein
P3D	30205_57	3	7,568,111	Spo22384.1	Ankyrin repeat family protein
P3D	30206_67	3	7,568,422	Spo22384.1	Ankyrin repeat family protein
P3D	30671_114	3	8,620,811	Spo10329.1	Tudor/PWWP/MBT superfamily protein
P3D	30799_148	3	88,465,472	Spo22910.1	Kinase family protein
P3D	30800_38	3	88,465,472	Spo22910.1	Kinase family protein
P3D	31487_100	4	100,381,644	Spo16149.1	RNA binding protein-like protein
P3D	31489_83	4	100,381,644	Spo16149.1	RNA binding protein-like protein
P3D	31747_46	4	10,380,625	Spo14207.1	Dynamin related protein 4C
P3D	31749_105	4	10,380,625	Spo14207.1	Dynamin related protein 4C
P3D	32854_93	4	118,696,771	Spo19914.1	Eukaryotic peptide chain release factor subunit 1-3
P3D	33007_60	4	119,528,399	Spo19816.1	Pre-mRNA-processing 40A-like protein
P3D	33559_113	4	16,421,490	Spo13323.1	Serine/threonine-protein kinase Haspin, putative
P3D	34035_132	4	26,810,902	Spo08574.1	Lipoxygenase (1.13.11.-)
P3D	34036_132	4	26,818,569	Spo08575.1	Lipoxygenase (1.13.11.-)

P3D	34039_42	4	26,819,040	Spo08575.1	Lipoxygenase (1.13.11.-)
P3D	34039_87	4	26,818,995	Spo08575.1	Lipoxygenase (1.13.11.-)
P3D	34117_137	4	28,325,117	Spo23961.1	Unknown protein
P3D	34118_143	4	28,325,294	Spo23961.1	Unknown protein
P3D	34242_136	4	31,387,745	Spo20667.1	Carbohydrate-binding X8 domain protein
P3D	34271_61	4	32,550,978	Spo25836.1	Pentatricopeptide repeat-containing protein, putative
P3D	35060_72	4	54,436,791	Spo19617.1	F23A5.27 isoform 5
P3D	35061_75	4	54,436,791	Spo19617.1	F23A5.27 isoform 5
P3D	35072_118	4	54,716,214	Spo21108.1	Ran-binding family protein
P3D	35073_91	4	54,716,214	Spo21108.1	Ran-binding family protein
P3D	35144_0	4	5,610,249	Spo00664.1	RNA-binding KH domain protein
P3D	35192_136	4	5,687,454	Spo00688.1	Receptor-like kinase
P3D	37772_125	5	12,402,600	Spo05255.1	Leo1-like family protein
P3D	37773_10	5	12,402,647	Spo05255.1	Leo1-like family protein
P3D	38605_0	5	22,806,525	Spo10884.1	U-box domain-containing protein 25 (6.3.2.-) (Plant U-box protein 25)
P3D	38677_87	5	2,430,492	Spo02295.1	Probable WRKY transcription factor 21 (WRKY DNA-binding protein 21)
P3D	38765_60	5	253,906	Spo17658.1	Transmembrane protein, putative
P3D	38846_0	5	2,660,648	Spo02382.1	Galacturonosyltransferase 6, putative
P3D	39017_113	5	2,866,480	Spo02281.1	(Histone acetyltransferase gcn5, putative) (2.3.1.48)
P3D	39133_63	5	303,261	Spo17581.1	Cap-binding protein 20
P3D	39624_108	5	41,089,616	Spo14565.1	Late embryogenesis abundant protein-like
P3D	39626_18	5	41,090,038	Spo14565.1	Late embryogenesis abundant protein-like

P3D	39928_138	5	45,792,763	Spo21150.1	Acetyltransferase component of pyruvate dehydrogenase complex (2.3.1.12)
P3D	39928_29	5	45,792,872	Spo21150.1	Acetyltransferase component of pyruvate dehydrogenase complex (2.3.1.12)
P3D	40321_114	5	54,817,921	Spo21272.1	RNA-binding protein 45
P3D	40625_79	5	674,700	Spo17599.1	pumilio 23
P3D	40735_0	5	694,638	Spo17602.1	Ribonucleases P/MRP protein subunit POP1
P3D	41177_60	6	11,745,075	Spo16849.1	NAD(P)-binding Rossmann-fold superfamily protein
P3D	41186_0	6	12,079,129	Spo04996.1	(Oligopeptidase A, putative) (3.4.24.70)
P3D	41452_59	6	17,027,317	Spo12191.1	40S ribosomal protein S4
P3D	41504_103	6	17,811,377	Spo06606.1	Probable zinc metalloprotease EGY2, chloroplastic (3.4.24.-) (Protein ETHYLENE-DEPENDENT GRAVITROPISM-DEFICIENT AND YELLOW-GREEN 2) (Precursor)
P3D	41661_12	6	19,763,961	Spo15265.1	NADP-dependent D-sorbitol-6-phosphate dehydrogenase
P3D	42094_0	6	25,976,357	Spo23503.1	Multidrug resistance protein ABC transporter family protein
P3D	42099_16	6	25,978,488	Spo23503.1	Multidrug resistance protein ABC transporter family protein
P3D	42181_69	6	2,643,950	Spo08906.1	Pyruvate decarboxylase family protein
P3D	42252_95	6	2,698,203	Spo08906.1	Pyruvate decarboxylase family protein
P3D	42409_119	6	28,347,889	Spo07721.1	Double-stranded RNA-binding protein 1 (dsRNA-binding protein 1)

P3D	42415_26	6	28,362,409	Spo07722.1	Q2QLY5 5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase 1 (2.1.1.14) (Cobalamin-independent methionine synthase 1)
P3D	42715_147	6	32,370,490	Spo26891.1	Superoxide dismutase [Cu-Zn] (1.15.1.1)
P3D	42737_145	6	32,402,180	Spo26944.1	Cytochrome P450, putative
P3D	42767_70	6	32,519,350	Spo26906.1	Bile acid:sodium symporter family protein
P3D	42952_0	6	34,212,703	Spo18799.1	(Gamma-glutamylcysteine synthetase) (6.3.2.2)
P3D	42959_132	6	34,218,828	Spo18799.1	(Gamma-glutamylcysteine synthetase) (6.3.2.2)
P3D	43139_149	6	35,410,747	Spo09330.1	Transducin/WD-like repeat-protein
P3D	43229_0	6	36,336,517	Spo14726.1	(Cytochrome P450, putative) (1.14.14.1)
P3D	43483_45	6	38,633,662	Spo00709.1	Heat shock transcription factor
P3D	43489_42	6	38,651,061	Spo00707.1	myb domain protein 3r-4
P3D	43496_89	6	38,741,448	Spo00700.1	Mitochondrial substrate carrier family protein
P3D	43500_132	6	38,755,116	Spo00700.1	Mitochondrial substrate carrier family protein
P3D	43721_16	6	40,661,995	Spo26015.1	Endoglucanase (3.2.1.4)
P3D	43722_133	6	40,687,330	Spo25927.1	IQ-domain 17, putative
P3D	43724_99	6	40,687,330	Spo25927.1	IQ-domain 17, putative
P3D	44122_1	6	43,822,462	Spo23392.1	Mitochondrial carrier protein

P3D	44132_19	6	43,909,231	Spo23388.1	Probable ADP-ribosylation factor GTPase-activating protein AGD8 (ARF GAP AGD8) (Protein ARF-GAP DOMAIN 8) (AtAGD8)
P3D	44154_56	6	44,253,924	Spo23374.1	E3 ubiquitin-protein ligase RNF14
P3D	44154_69	6	44,253,937	Spo23374.1	E3 ubiquitin-protein ligase RNF14
P3D	44175_144	6	44,490,636	Spo23366.1	Homeobox-leucine zipper protein ATHB-8 (HD-ZIP protein ATHB-8) (Homeodomain transcription factor ATHB-8)
P3D	44179_24	6	44,496,133	Spo23246.1	Small ubiquitin-related modifier (SUMO)
P3D	44474_125	6	7,040,639	Spo04005.1	U-box domain-containing protein 8
P3D	44474_59	6	7,040,573	Spo04005.1	U-box domain-containing protein 8
P3D	52966_144	6	45,522,356	Spo11415.1	Stress-inducible protein, putative
P3D, perMarker	22850_134	2	35,442,434	Spo25147.1	Unknown protein
P3D, perMarker	22852_76	2	35,442,434	Spo25147.1	Unknown protein
P3D, perMarker	22857_54	2	35,444,258	Spo25147.1	Unknown protein
P3D, perMarker	24710_141	2	54,179,913	Spo23859.1	Mitochondrial import inner membrane translocase subunit Tim17/Tim22/Tim23 family protein
P3D, perMarker	35965_139	4	7,461,542	Spo11988.1	Saccharopine dehydrogenase
P3D, perMarker	36035_144	4	7,529,193	Spo11912.1	Regulator of chromosome condensation (RCC1) family with FYVE zinc finger domain
P3D, perMarker	42188_0	6	26,519,439	Spo26833.1	RNA helicase family protein
P3D, perMarker	43139_147	6	35,410,749	Spo09330.1	Transducin/WD-like repeat-protein
P3D, perMarker	44004_89	6	43,055,851	Spo09708.1	Haloacid dehalogenase-like hydrolase

P3D, perMarker	44006_90	6	43,055,851	Spo09708.1	Haloacid dehalogenase-like hydrolase
perMarker	18253_80	1	23,243,114	Spo05903.1	F-box family protein
perMarker	19686_116	1	38,745,477	Spo25730.1	Pentatricopeptide repeat-containing protein
perMarker	19686_116	1	38,745,477	Spo25771.1	SNF1-related protein kinase regulatory subunit gamma-1-like protein
perMarker	19895_47	1	40,433,857	Spo10712.1	Nucleobase-ascorbate transporter 7
perMarker	20428_123	1	44,730,648	Spo09957.1	Hydroxyproline-rich glycoprotein family protein, putative
perMarker	20429_38	1	44,730,648	Spo09957.1	Hydroxyproline-rich glycoprotein family protein, putative
perMarker	21406_95	1	64,715	Spo02922.1	Peroxisomal membrane 22 kDa (Mpv17/PMP22) family protein
perMarker	22066_109	2	19,582,976	Spo04750.1	(Nuclear transcription factor Y subunit B) (Nuclear transcription factor Y subunit B2)
perMarker	22068_147	2	19,582,866	Spo04750.1	(Nuclear transcription factor Y subunit B) (Nuclear transcription factor Y subunit B2)
perMarker	23027_134	2	3,805,442	Spo12249.1	RING/U-box superfamily protein, putative
perMarker	23134_58	2	39,622,079	Spo01934.1	UDP-Glycosyltransferase superfamily protein
perMarker	23155_93	2	40,008,560	Spo14439.1	Histone H3
perMarker	23937_86	2	48,648,516	Spo00422.1	ENTH/VHS/GAT family protein
perMarker	23951_12	2	4,882,570	Spo10207.1	(Germin-like protein) (Precursor)
perMarker	23952_42	2	4,882,770	Spo10207.1	(Germin-like protein) (Precursor)
perMarker	23963_76	2	48,855,023	Spo00418.1	Phospholipase D (3.1.4.4)

perMarker	24275_0	2	50,997,283	Spo01622.1	Ubiquitin conjugating-like enzyme family protein
perMarker	24284_6	2	51,045,598	Spo01720.1	Tubulin gamma chain
perMarker	24289_62	2	51,133,139	Spo01629.1	Probable indole-3-acetic acid-amido synthetase GH3.5 (6.3.2.-) (Auxin-responsive GH3-like protein 5) (OsGH3-5)
perMarker	24294_15	2	51,212,634	Spo24373.1	DEAD-box ATP-dependent RNA helicase-like protein
perMarker	24484_0	2	52,272,488	Spo23703.1	BEACH domain LvsC-like protein, putative
perMarker	24487_0	2	52,280,683	Spo23703.1	BEACH domain LvsC-like protein, putative
perMarker	24511_8	2	52,364,078	Spo23665.1	(DNA binding protein) (MYB transcription factor)
perMarker	24765_89	2	54,639,882	Spo17895.1	Beta-D-xylosidase
perMarker	25127_28	2	56,656,947	Spo21681.1	(Ferredoxin/thioredoxin reductase subunit A (Variable subunit) 1) (Similarity to ferredoxin-thioredoxin reductase variable chain)
perMarker	25162_8	2	56,878,757	Spo21764.1	(Protein arginine n-methyltransferase, putative) (2.1.1.125)
perMarker	25431_75	2	58,860,976	Spo01178.1	(Receptor protein kinase, putative) (1.3.1.74) (2.7.11.25)
perMarker	25436_16	2	58,889,189	Spo01124.1	Adenylate kinase
perMarker	25436_24	2	58,889,197	Spo01124.1	Adenylate kinase
perMarker	25437_141	2	58,889,197	Spo01124.1	Adenylate kinase

perMarker	25437_149	2	58,889,189	Spo01124.1	Adenylate kinase
perMarker	26391_148	3	11,201,274	Spo08335.1	Fip1 [V]-like protein
perMarker	26394_5	3	11,201,383	Spo08335.1	Fip1 [V]-like protein
perMarker	26597_53	3	12,731,426	Spo22859.1	SacI domain-containing protein / WW domain-containing protein isoform 1
perMarker	26599_101	3	12,731,426	Spo22859.1	SacI domain-containing protein / WW domain-containing protein isoform 1
perMarker	26903_132	3	16,213,630	Spo24334.1	Sulfotransferase (2.8.2.-)
perMarker	26906_9	3	16,220,233	Spo24334.1	Sulfotransferase (2.8.2.-)
perMarker	31213_146	3	95,602,919	Spo12622.1	Unknown protein
perMarker	31213_80	3	95,602,853	Spo12622.1	Unknown protein
perMarker	31253_134	3	96,280,174	Spo09827.1	SAGA-associated factor 11
perMarker	31253_135	3	96,280,175	Spo09827.1	SAGA-associated factor 11
perMarker	31541_147	4	100,891,594	Spo16204.1	Ubiquitin system component Cue protein, putative
perMarker	33871_81	4	21,136,142	Spo02469.1	Topless-related protein 3
perMarker	33962_87	4	25,149,405	Spo04659.1	Unknown protein
perMarker	33968_36	4	25,447,636	Spo17936.1	DNAJ
perMarker	34242_83	4	31,387,798	Spo20667.1	Carbohydrate-binding X8 domain protein
perMarker	34332_85	4	34,713,850	Spo26360.1	basic leucine-zipper 42
perMarker	34498_36	4	40,261,080	Spo02443.1	(Kinase, putative) (2.7.11.25)
perMarker	34498_37	4	40,261,081	Spo02443.1	(Kinase, putative) (2.7.11.25)
perMarker	34762_104	4	47,845,972	Spo20479.1	(Phosphatidylserine decarboxylase, putative) (4.1.1.65)
perMarker	36212_42	4	78,322,265	Spo22539.1	Expressed protein
perMarker	36553_140	4	84,239,999	Spo27188.1	Glycolipid transfer protein (GLTP) family protein

perMarker	36554_95	4	84,239,999	Spo27188.1	Glycolipid transfer protein (GLTP) family protein
perMarker	37433_86	4	98,770,437	Spo26724.1	Kinesin-like protein
perMarker	37435_133	4	98,770,437	Spo26724.1	Kinesin-like protein
perMarker	38885_75	5	2,676,049	Spo02379.1	(Glutathione reductase, putative) (1.8.1.7)
perMarker	38886_118	5	2,676,049	Spo02379.1	(Glutathione reductase, putative) (1.8.1.7)
perMarker	39759_146	5	42,767,871	Spo30008.1	ARF guanine-nucleotide exchange factor GNOM protein
perMarker	40173_114	5	5,143,287	Spo00286.1	HAUS augmin-like complex subunit-like protein
perMarker	42189_110	6	26,519,627	Spo26833.1	RNA helicase family protein
perMarker	42416_5	6	28,362,655	Spo07722.1	Q2QLY5 5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase 1 (2.1.1.14) (Cobalamin-independent methionine synthase 1)
perMarker	42423_18	6	28,430,761	Spo07695.1	Plant protein of unknown function (DUF639)
perMarker	42767_64	6	32,519,356	Spo26906.1	Bile acid:sodium symporter family protein
perMarker	42769_96	6	32,540,672	Spo26907.1	Uncharacterised protein family Ycf49
perMarker	43458_141	6	38,350,300	Spo00724.1	GRAS family protein
perMarker	43458_38	6	38,350,197	Spo00724.1	GRAS family protein
perMarker	43636_80	6	39,917,492	Spo11593.1	Histone H4
perMarker	43739_4	6	40,798,438	Spo26007.1	Ubiquitin carboxyl-terminal hydrolase (3.4.19.12)

perMarker	43829_58	6	41,499,476	Spo25880.1	(2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein) (Hyoscyamine 6-dioxygenase hydroxylase, putative)
perMarker	43831_125	6	41,505,741	Spo25879.1	myb domain protein 65
perMarker	44061_0	6	43,411,046	Spo23415.1	ABC transporter family protein
perMarker	44066_80	6	43,426,953	Spo23320.1	PWWP domain-containing family protein
perMarker	44093_13	6	43,539,878	Spo23408.1	Disease resistance protein
perMarker	44095_138	6	43,540,150	Spo23408.1	Disease resistance protein
perMarker	44185_68	6	44,519,636	Spo23365.1	Transcription factor jumonji family protein
perMarker	44190_115	6	44,602,354	Spo23244.1	Glycerol-3-phosphate acyltransferase
perMarker	44257_91	6	45,185,282	Spo11390.1	SH3 domain-containing protein 1
perMarker	44258_61	6	45,185,282	Spo11390.1	SH3 domain-containing protein 1
perMarker	50006_58	3	1,999,745	Spo12868.1	Thioredoxin-related family protein