IDENTIFICATION OF GENOMIC REGIONS ASSOCIATED WITH COLOR-RELATED TRAITS IN DURUM WHEAT

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

DOCTOR OF PHILOSOPHY

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ABSTRACT

Durum wheat (*Triticum turgidum* L. *ssp*. *durum* Desf.) is one of the main crops for human consumption, and it is an essential ingredient for pasta production. The color of pasta can range from bright yellow to dim brown. Pasta brightness and yellowness are important quality indicators for producers and customers. Releasing durum wheat cultivars capable of producing pasta products with high yellow color can be an important goal for breeders. Yellow pigment content (YPC) is one of the important components that affect pasta color. The yellow color of pasta is not necessarily dictated by the presence of YPC; soluble brown pigment (SBP) and enzymes such as polyphenol oxidase (PPO) and peroxide (POD) are other factors that contribute to pasta color. Marker-assisted selection (MAS) can accelerate the selection process for breeding programs that develop new durum wheat cultivars with high yellow color. This dissertation used quantitative trait loci (QTL) mapping to identify QTL for these color-related traits. Two populations, each with 192 recombinant inbred lines (RILs), were developed from crosses between Joppa and D12118 (Population one [POP1]) and Mountrail and Carpio (Population two [POP2]). The phenotyping of these traits was carried out in two locations in North Dakota, USA. Genotyping of the RIL populations was conducted using the wheat Illumina iSelect 90K SNP assay. There were significant phenotypic differences among the genotypes for all traits. In POP1, entry lines 32 and 78 with both high YPC and low SBP, and in POP 2 entry lines 1, 82, and 101 with high YPC could be excellent sources for improving the yellow color in durum wheat. In this study, 31 Additive QTL (A-QTL) and 370 minor digenic epistatic QTL (DE-QTL) associated with all four traits were identified. In Pop1, the most significant A-QTL were detected on Chromosomes 5A (*D1.SBP5A.ndsu*) for SBP and 5B (*D1.YPC5B.ndsu*) and 6B (*D1.YPC6B.ndsu)* for YPC. In POP2, major A-QTL were found on chromosomes 7A (*D2.YPC7A.ndsu*), 2A (*D2.PPO2A.ndsu*), and 3A (*D2.POD3A.ndsu*) for YPC, PPO, and POD, respectively. The information provided in the current study could be employed with MAS to increase selection efficiency and improve the color of durum wheat.

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DEDICATION

This dissertation is dedicated to my parents, who have always been supportive of me throughout my life.

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LIST OF ABBREVIATIONS

AM	Association Mapping
A-QTL	Additive QTL
DE-QTL	Digenic Epistatic
GS	Genomic Selection
GWAS	Genome-wide Association Study
LOD	Logarithm of the Odds
LOX	Lipoxygenase
MAS	Marker-assisted Selection
PDS	Phytoene Desaturase
POD	Peroxidase
POP1	Population One
POP2	Population Two
PPO	Polyphenol Oxidase
PSY	Phytoene Synthase
PVE	Phenotypic Variation Explained
QTL	Quantitative Trait Loci
RIL	Recombinant Inbred Line
SBP	Soluble Brown Pigment
SNP	Single Nucleotide Polymorphisms
SSD	Single Seed Descent
TWT	Test Weight
YI	Yellow Index
YPC	Yellow Pigment Content
ZDS	Zeta-carotene Desaturase

CHAPTER 1. LITERATURE REVIEW

Evolution and Domestication of Durum Wheat

Almost 50% of worldwide energy food sources come from the cereal plants such as rice (Oryza sativa), maize (Zea mays), and wheat (*Triticum aestivum L*.) (HTTP ://www.fao.org/3/CA179 6EN/ca179 6en.pdf). However, the most critical crop is wheat (HTTP ://www.fao.org/asset s/infog raphi cs/FAO-Infog raphi c-wheat -en. pdf). There are eight wheat classes that are grown in the US: hard red winter (HRW), hard red spring (HRS), soft red winter (SRW), durum (*Triticum turgidum* L. ssp. *durum* Desf.), white (soft white winter (SWW) and club), hard white winter (HWW), mixed, and unclassified (Delwiche and Norris, 1993). Among all the wheat classes, durum wheat is more tolerant to drought than hexaploid bread wheat. Durum wheat has the hardest kernels, a unique amber color, and a quality nutrient that makes it the best raw material for manufacturing end-pasta products (Dexter and Edwards, 1998; Elias and Manthey, 2005). About 6–8% of the world's total wheat production is durum wheat (Troccoli et al., 2000). Durum wheat has three subclasses, based on vitreous kernel content, hard amber ($\geq$75%), amber (between 60 to 74%), and durum wheat (<60%) (Elias and Manthey, 2005). Durum wheat belongs to the grass family, Poaceae and the Tririceae tribe. Durum wheat is an allotetraploid (2n=4x=28, genome AABB) that was developed from hybridization between *Triticum urartu* (A genome) And A. speltoides (B genome) as the donor species. This hybridization was followed by chromosome doubling and domestication (Levy and Feldman, 2002).

Domestication is the process of humans selecting suitable and desirable traits from wild crops and animals. Human civilization came from the domestication of animals and plants (Gepts, 2004). The geographic area where domestication began is referred to crop`s center of origin. The area known as Fertile Crescent, which reaches from north of the Persian Gulf to the east side of the Mediterranean Sea, and stretches south into northern Africa, is the origin of the wheat and durum wheat. *Triticum durum*, is a wild relative of durum wheat, which still grows in the middle East (Abbo et al., 2010; Zhang et al., 2014).

The evolution and domestication of durum wheat is very complex, and happened in several steps. Like other cereal crops, wheat originated in the eastern area of the Fertile Crescent, and it was grown from wild grasses (Bozzini, 1988). All kinds of wheat are members of the *Hordeae* tribe and are classified in the *Triticum* genus (Bozzini, 1988). Almost 500,000 years ago, the hybridization of two diploid

ancestors, *Triticum Urartu* (AA) and *Aegilops speltoides* (SS), made tetraploid species *Triticum dicoccoides* (AASS genome). Approximately 10,000 years ago, farmers started to domesticate tetraploid from wild emmer wheat (WEW) (*Triticum dicoccum*) to domesticated emmer wheat (DEW) (T. *turgidum ssp. dicoccum*) with an AABB genome (Dubcovsky and Dvorak, 2007; Flint-Garcia, 2013; Maccaferri et al., 2019). Durum wheat appeared for the first time around 6,500-7,000 years ago. About 2,000 years ago durum wheat became the main crop in the Fertile Crescent. With continuing domestication and breeding of durum wheat, the WEW and DEW changed to durum wheat landraces (DWL), and from DWL to modern cultivated durum wheat (Macaferri et al., 2019).

Wild and modern varieties of durum wheat have several differences in phenotypical, genetic, and physiological characters. These changes are explained as domestication syndrome. A few of these changes include; the modern varieties have lost natural seed diffusion, the male and female organs have synchronous flowering between each other, modern varieties have a more vigorous plant, larger fruits or grains, have less seed dormancy, and have increased apical dominance (Uauy et al., 2006; Steffenson et al., 2007). Although, some traits have been reduced compared to the wild species, such as disease resistance (Flint-Garcia, 2013; Ellis et al., 2014). Research studies about durum wheat ancestors indicated that the wild ancestor of durum wheat had a very fragile spikelet, which was due to allele q. However, the domesticated ancestor of durum had allele Q, which was associated with a non-brittle rachis (Vaughan et al., 2007). At the time of domestication, the first farmers were the first breeders, and based on the phenotypic characteristics of the plant, they chose the high-performance plants instead of low-performance ones. In the wild ones, the allele Q did not exist, but the farmers' selection in post-domestication resulted in the emergence of this allele (Vaughan et al., 2007). These significant changes couldn't be achieved if the perfect management did not apply. Modified ecosystems in conjunction with changes in soil fertility are the critical management strategies for this goal.

With continuing breeding of modern durum wheat cultivars, a significant reduction in the genetic diversity of durum wheat occurred. Wild durum wheat has more genetic diversity than modern durum wheat cultivars (Dwivedi et al., 2016; Macaferri et al., 2019). Phenotypic variation has been managed in the crop domestication period. They were reducing genetic diversity related to wild species and achieving

better quality in crop domestication. Nowadays, diversity is influenced by domestication and breeding programs (Flint-Garcia, 2013).

U.S. Commercialization of Durum Wheat

As time started to settle, and people started to practice agricultural activity, wheat spread to the other continents and countries from the Middle East. Approximately 7,000 years B.C., wheat reached the Iberian Peninsula (Nazco et al., 2014). Durum wheat entered the Iberian Peninsula and North Africa from the southern part of Italy.

Researchers have studied genetic similarities of wheat grown in different regions, and they explained that Spain and Portugal could have been a migration route for spreading durum wheat to the Iberian Peninsula. During migration, the different environments, natural selection, and human selection lead to many varieties from landraces, and they observed similar adaptations in other agro-ecological regions. Farmers typically planted different varieties of durum wheat, especially in Italy, until the green revolution. After that time, the adapted durum wheat, which had been distributed throughout the world, became part of the main crop of each area. (Nazco et al., 2014).

The U.S. Department of Agriculture (USDA) started collecting and distributing the early germplasm such as Algerian Flint, Turkish Flint, Arnautka, and Syrian Spring to the farmers in approximately 1850 (Joppa and Williams, 1988). Durum wheat was used to feed livestock from 1850 to 1870, because producers had difficulties growing most varieties in developing conditions, and the milling was difficult because of the hard-vitreous kernels of durum wheat. The first state that grew durum wheat was Texas, which imported durum wheat from Nicaragua. The high yield, disease resistance, and high quality of durum wheat was exciting for farmers; however, the difficulties with milling caused durum wheat to disappear from Texas by 1890. The USDA had begun to study wheat varieties for high yield and resistance to rust in 1894. After that, they found more than 1,000 varieties of durum wheat from all parts of the world and started growing them in nurseries in Kansas and Maryland in 1895-1897. The best 200 varieties were then selected for further study (Royo et al., 2009). Several states started to gather a collection of durum wheat varieties, and North Dakota (ND) was one of them. Researchers from ND found tetraploid wheat (durum) to replace hexaploid wheat, such as spring wheat, which had more yield and

more resistance to leaf rust. Mark Carleton (known as the father of durum wheat in the northern plains in 1890) collected several grains from Russia and brought them to the United States.

Also, Carleton had kinds of wheat named 'others,' which were classes of wheat that Russians did not want to grow. The durum wheat, which farmers called 'goose wheat' was too hard for milling; however, due to its resistance to leaf rust and other diseases, Carlton made this goose wheat acceptable for farmers. This resulted in the northern region of the Great Plains becoming the largest producer of durum wheat since 1910. At that time, ND produced more than 75% of United States durum wheat, exporting it to Europe and other countries. Climate conditions, prices of hard spring wheat and durum wheat, and susceptibility to leaf rust affect the amount of durum wheat production (Royo et al., 2009).

In 1929, USDA began the first durum wheat breeding program in Langdon, ND (Bozzini, 1988; Sissons, 2012). The breeders used various cultivars from North Africa, Russia, Greece, and other areas to improve quality and resistance to diseases of durum wheat in the Great Plains (Bozzini, 1988; Sissons, 2012).

Most breeding programs before the late 1960s were focused on resistance to stem rust, which severely decreased durum production (Bozzini, 1988; Sissons, 2012). In 1951, breeders, pathologists, and geneticists searched for valuable germplasm to enhance resistance to the disease. As a result, Khapli emmer, PI94701, and some derivatives were developed to have resistance to the disease; these cultivars turned out to have at least nine different loci for resistance on stem rust (Bozzini, 1988). Researchers crossed these valuable germplasms to susceptible cultivars and tested their disease resistance with artificial disease inoculation (Bozzini, 1988). Eventually, they could breed novel resistant cultivars (Wells and Lakota) to the stem rust with the modified pedigree method and the introduction of resistance genes (*Sr7a*, *Sr13*, and *Sr14*) (Bozzini, 1988). However, due to 30 years of struggling with stem rust these cultivars did not have good quality traits (Bozzini, 1988). Along with resistance in durum wheat, breeding programs developed useful cultivars with increased yellow pigment content, lodging resistance, shattering resistance, reduced plant height, and early maturity by crossing germplasms (Bozzini, 1988). In 1963, Lebsock transferred semi-dwarf gene *Rht-1* to durum wheat from hexaploid wheat to reduce plant height. During the 1970s and 1980s, durum breeders in the United States, Canada,

and Italy improved gluten strength by selecting the favorable γ-gliadin genotype (LMW-1 and LMW-2) with polyacrylamide gel electrophoresis (Bozzini, 1988; Liu et al., 1996).

With modern breeding programs that include only a few elite cultivars in their crossing programs, the genetic-based wheat germplasm has narrowed (Pascual et al., 2020). Researchers have adopted various methods to solve narrowed genetic diversity within durum wheat breeding, including double haploid, genetic transformation, marker-assisted selection (MAS) (Sissons, 2012), and using landraces in their breeding programs (Salsmen et al., 2021). Landraces are one of the essential resources for enhancing genetic diversity in breeding programs (Herbabdez-Espinosa et al., 2020).

Double haploid can be an advantage for incorporation of quantitative traits which cannot be selected from the conventional breeding programs (Sissons, 2012). Since previous breeding methods reduced the genetic diversity in durum wheat, researchers have imported valuable genes from other related species, including powdery mildew resistance gene (*Pm13*), leaf rust resistance gene (*Lr19*), and yellow pigment gene (*Y*) (Sissons, 2012).

Due to similarities in their genome, interspecific crosses are commonly made between hexaploid wheat and durum wheat to incorporate useful traits (Sissons, 2012). With these research efforts, current durum wheat has improved gluten content, yellow pigment content (YPC), and high disease resistance (Sissons, 2012).

In order to calculate the market value of durum wheat, factors such as grain quality and the end-use products must be considered (Johnson et al., 2019). The elements that influence quality can have a negative impact on the market value. Diseases, pests, and environmental issues can all have an impact on the quality of durum wheat (Kubalakova et al., 2005). Among the most important end-use quality traits for durum wheat are color, protein quantity and quality, weather damage (falling number), virtuousness, and visual appearance (Johnson et al., 2019).

Molecular Characterization of Durum Wheat

Durum wheat (*Triticum turgidum* L. ssp. *durum* Desf.) is tetraploid (2n=4x=28, AABB genome), which originated from intergeneric polyploidization between *T. urartu* (2n=2x=14, AA genome) and *Aegilops speltoides* (2n=2x=14, BB genome) (Maccaferri et al., 2015). The construction of the bacterial artificial chromosomes (BAC) library characterized the genome size of durum as 13,000 Mb

(Cenci et al., 2003). Most molecular genetic research of durum wheat has involved hexaploid wheat (bread wheat) since durum wheat shares the genetic information of two A and B genomes with hexaploid wheat (Maccaferri et al., 2015; Holtz et al., 2016; Mengistu et al., 2016). A high-quality reference sequence of hexaploid wheat is available at Internal Wheat Genome Sequencing Consortium, which also can be used for durum wheat (Mengistu et al., 2016). To improve durum wheat by MAS, researchers have developed molecular markers based on the studies of hexaploid wheat by considering differences in durum wheat polymorphisms with hexaploid wheat (Maccaferri et al., 2015; Holtz et al., 2016; Mengistu et al., 2016; Baloch et al., 2017).

To identify the genetic diversity of durum wheat, researchers had developed various molecular markers such as microsatellite or simple sequence repeat (SSR) and Diversity Arrays Technology (DArT) markers (Hu et al., 2015; Holtz et al., 2016). However, due to disadvantages inefficiency of these markers, single nucleotide polymorphisms (SNP) has become widely utilized globally with low cost, high-throughput, and high polymorphisms (Hu et al., 2015; Maccaferri et al., 2015; Mengistu et al., 2016). Maccaferri et al. (2015) assembled the high-density consensus map from 13 independent bi-parental durum wheat populations (12 recombinant inbred lines [RILs] and one double haploid), and 1,928 lines genotyped with Illumina 9K and 90K wheat SNP arrays. These populations were derived from elite x elite, elite x cultivated emmer, and elite x wilder emmer (ancestor) to create a genetic reference map, identify novel traits, and solve genetic diversity limitations. The 1,773 RILs were used for the construction of a framework map with the identification of anchor markers, which were grouped into 14 linkage groups. A final consensus map was developed with the framework map, including 13,760 uniquely mapped markers from single component populations and 30,144 markers. This map was created using 5,279 unique recombination events (377 per chromosome), and it covered 2,631 cM with an average intermarker distance of 0.087 cM, or 11 markers per cM (Maccaferri et al., 2015).

In order to improve durum wheat, characterized molecular markers have been used to detect agricultural traits (Haile et al., 2013; Hu et al., 2015; N'Diaye et al., 2017). Haile et al. (2013) focused on quantitative trait loci (QTL) mapping for stem-solidness locus *SSt1* since solid-stemmed wheat cultivars alleviate wheat stem sawfly damage by conferring a physical barrier to restrict larval movement. They identified a major QTL on chromosome 3BL to improve the effect of *SSt1*. Hu et al. (2015) performed

association mapping (AM) for agronomic traits including plant height (PH), effective spikes (ES), grain weight per plant (GWP, g), etc., based on linkage disequilibrium (LD) from 150 durum wheat genotypes. The research identified several available SNP markers on the genome, and specific chromosome regions locate these agronomic traits (Hu et al., 2015). Also, N'Diaye et al. (2017) developed a high-density consensus map related to pasta color in elite durum wheat by haplotype-based association analysis. Due to the demand for bright yellow color pasta, the global breeding programs have made improving yellow pigment in durum wheat, one of their major objectives. *Lpx-B1* marker that is associated with yellow pigment was used for improving color in durum wheat (N'Diaye et al., 2017). Association mapping detected the locus hap_4B_6 on chromosome 4B, a potential candidate for the *Lpx-B1* gene (N'Diaye et al., 2017). Using the high-density molecular map, research could identify valuable markers for agronomic traits and the location on chromosomes of potential markers for durum wheat MAS.

Genetics of Durum Wheat Agronomic Traits

The most important agronomic traits in durum wheat are yield, test weight (TWT), lodging, plant height, leaf diseases, and falling number. These agronomic traits are controlled mainly by genotype and genotype and environment interaction, as they are quantitative traits (McCartney et al., 2005; Li et al., 2016).

Yield

One of the most important agronomic traits in crops is yield and yield components (hosseinirad et al., 2013). Yield is one the most complex traits in durum wheat that is controlled by many genes throughout the genome (Mohsin et al., 2009; Kahrizi et al., 2010). Modern breeding programs achieved high grain yield in durum wheat (Elias et al., 2021), however, it resulted in reduced genetic diversity (Dwivedi et al., 2016). The use of durum wheat landraces can improve the genetic diversity, yield component traits, disease resistance, and the quality of end-use pasta products (Salsmen et al., 2021).

Test Weight

Another essential agronomic trait for durum wheat is TWT, which is necessary for marketing. Test weight is an amount of the bulk density of wheat grain articulated in pounds per bushel (lb/bu), kilograms per cubic meter (kg m-3), or kilograms per hectoliter (kg/hl). In the U.S., durum wheat is graded based on the TWT, with a minimum TWT of 60.0, 58.0, 56.0, and 54.1 lb/bu (74.8, 72.3, 70.0, and 67.5 kg/hl,

respectively) required to achieve the U.S., Nos. 1, 2, 3, and 4, respectively. Test weight has been reported to be highly correlated with kernel shape and kernel size (Yamazaki and Briggle, 1969). Genotype by environment interaction significantly impacts TWT since it is a quantitative trait in nature (Rharrabti et al., 2003b). Researchers found 11 putative QTL related to TWT on chromosomes 1AL, 1BL, 2, 3BS, 3BL, 5, 6, and 7. Five of these QTL were found exclusively in one environment, three were found in two environments, and three were found in five or more environments. The majority of these QTL were found to be co-located with thousand kernel weight (TKW) QTL (Graziani et al., 2014). A total of five QTL for TWT were discovered by Patil et al. (2013). An early generation selection strategy to improve TWT and TKW has been proposed that utilizes a QTL on chromosome 2A between the marker intervals Xgwm71.2–Xubc835.4.

Genetics of Durum Wheat End-use Quality Traits

In addition to yield and TWT, other important quality characteristics in durum breeding programs are grain weight (size), percentage of hard vitreous kernels, grain protein, milling yield, ash, YPC, semolina yellowness, gluten quality, falling number (FN), and finally end product quality, which is typically pasta that has been dried and cooked (Sissons et al., 2020). Johnson (2018) stated that high-quality durum wheat grain should have a high protein content, a high percentage of vitreous kernels, a high FN, and a bright yellow color to meet the comprehensive quality standards of millers, end-product producers, and consumers. Pasta products like spaghetti, macaroni, and vermicelli are made from durum wheat, which is the best raw material. In part, this is due to the comparatively high concentration of YPC, low levels of enzyme activities such as polyphenol oxidase (PPO) and peroxidase (POD), and high levels of protein found in durum wheat. In turn, semolina quality is determined by the grain quality, which can be controlled genetically as well as influenced by the growing environment. Semolina quality impacts pasta processing time and cooking quality (Rharrabti et al., 2003a).

Yellow Pigment Content

In addition to producing new durum cultivars with improved yield components and disease resistance, various breeding programs throughout the world are also prioritizing the development of high-quality traits such as color in tandem with the release of new durum cultivars (Battenfield et al., 2016). Pasta color is one of the most important quality characteristics in durum wheat that is utilized for pasta

production (Colassuno et al., 2019). Spaghetti color (yellowness, brightness, and redness) impact spaghetti consumer appeal, with bright and yellow spaghetti being chosen over red and darker spaghetti (Alzuwaid et al., 2021).

In determining the quality of durum wheat pasta, one of the most essential factors is its bright yellow color, in addition to cooking behavior or taste (Borrelli et al., 1999). The favored color of the pasta is yellow, with brown being undesirable. Pasta yellow color is due to an YPC, which is the most important component determining the color of pasta (Colasuonno et al., 2019). The end-use color is determined by the presence of a yellow pigment in the semolina, which is derived from grinding durum kernels. There are three parts to a durum wheat kernel: the germ (2-3%), the bran (14-16%), and the endosperm (81-84%) (Mousia et al., 2004). Semolina is obtained after removing the germ and the bran in the milling process. Bran is a rich source of nutrients, including fiber, antioxidants, and folates, among others. However, making pasta from whole-meal samples enriched with bran damages the color of the finished product (Alzuwaid et al., 2021) (Figure 1.1).

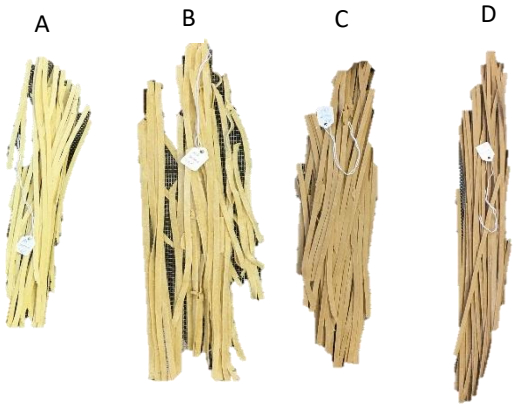

Figure 1.1. Increasing whole-meal in noodle samples, A- Control, B- 5% Whole-meal, C- 10% Whole-meal, and D- 20% Whole-meal.

To determine the color of durum wheat kernels and end-use pasta products, three elements of color are measured: the lightness of samples ranging from total black (0) to total white (100) (L*), greenness (-60) to redness (60) (a*), and blue (-60) to yellow (60) (b*) (Approved Method 76-31.01 of AACC). Because of the difficulty in producing semolina, using whole wheat flour to determine the pasta color has the benefit of saving time and being simpler to utilize (Sissons et al., 2020). There is 73 to 89% association between b* and color of whole wheat flour samples. The b* value, on the other hand, is lower

in the whole wheat flour samples due to the presence of bran in the samples. As stated by Alzuwaid et al. (2021), the color brightness (L*) of the samples was reduced to 74% when bran was added to the samples.

Durum wheat breeders must pay attention to two important characteristics: pigment content and semolina color. Yellow pigment content is the critical pigment in durum wheat (Ficco et al., 2014). A typical YPC ranges anywhere from 1.2 to 10 parts per million (ppm) of yellow pigment. The goal of pasta makers is to process their product with a bright yellow color (Singh et al., 2009). Semolina characteristics and enzyme activities, for example, may have an impact on these qualities. Multiple studies reveal a strong correlation between YPC in kernel and semolina's yellow index (YI). When it comes to YPC, stronger additive effects and low genotype by environment interaction make it highly heritable (86-95 percent) (Elouafi et al., 2001; Clarke et al., 2006; Van Hung and Hatcher, 2011; Colasuonno et al., 2017). According to Clarke et al., (2006), YPC levels are rising in new cultivars in most environmental circumstances, including cool and wet conditions. Lutein pigments, a group of carotenoids, are responsible for the vivid yellow color of durum wheat (Hessler et al., 2002).

Furthermore, the carotenoids serve as a significant aesthetic and nutritional health measure. In addition to their antioxidant capacity, all carotenoids include provitamin A activity, which protects against eye illnesses (Ribaya-Mercado and Blumberg, 2004). This decreases the chances of chronic degenerative diseases (Abdel-Aal et al., 2007). There has been an increase in interest in carotenoids recently because of their many health benefits for humans, including their ability to reduce oxidative damage associated with aging in both animal and human systems. Granado et al. (2003) demonstrated that lutein is one of the carotenoids that is most likely to benefit human health. Carotenoids, which are naturally occurring antioxidant molecules, are extremely significant in the nutritional and medicinal aspects of foods, as well as in the preservation of their freshness and storage stability (Shahidi, 2000). Also, carotenoids are an important nutritional ingredient of pasta products because they prevent oxidative damage to cellular membranes by scavenging peroxyradicals like those associated in some human diseases and aging processes (Bast et al., 1996). Ficco et al. (2014) reported that carotenoids are responsible for the antioxidant capacity and provitamin A activity of a number of nutrients, two of which are antioxidants. Similarly, anthocyanins also have therapeutic properties, and they are effective against

cancer, capillary fragility, oxidative liver damage, and various disorders in humans, among other benefits. Therefore, increasing carotenoids content in wheat-based foods is an important objective to pursue. In another study, Leenhardt et al. (2006) showed that lutein (followed by zeaxanthin) was the most abundant carotenoid in whole grains. Plants have a higher concentration of lutein than zeaxanthin due to lutein's role in photosynthesis (Mathis and Schenck, 1982).

In durum wheat, it is important to know the two distinct sets of genes determine the final color of pasta: the genes that express yellow pigment, and those that affect enzyme activity. According to numerus studies the YPC gene has been linked to several QTL throughout the durum wheat genome (Salsman et al., 2021). Each of these QTL affect the color differently. Chromosome 7 (telomeric regions on the long arm of the chromosome) is critical for color features in durum wheat. The genes on chromosomes 7A and 7B are responsible for more than half of the variation in color (Figure 1.2). In recent years, researchers have discovered many minor QTL responsible for durum wheat and bread wheat color in both arms of 7A and 7B (Zhang and Dubcovsky, 2008; Singh et al., 2009; Blanco et al., 2011; Roncallo et al., 2012).

Durum wheat's YPC can be controlled in two ways: effect of a single gene, or multiple genes have an influence (co-localization among a candidate gene) on the trait (Ficco et al., 2014). Carotenoid isomerase (*CRTISO*) is located on chromosome 1B and is involved in grain color (Salsman et al., 2021). Many minor QTL for YPC have been discovered in the durum wheat genome using new methodologies such as QTL mapping and AM. There are many QTL in durum wheat that affect both YPC and carotenoids, although a few solely affect one or the other (Blanco et al., 2011). On the other hand, some QTL exhibit a pleiotropy effect, as was reported by Just et al. (2009). Durum wheat had QTL for *α*-carotene accumulation and *β*-carotene activity, which they found to be controlled by QTL in 2A, 3B, and 7A.

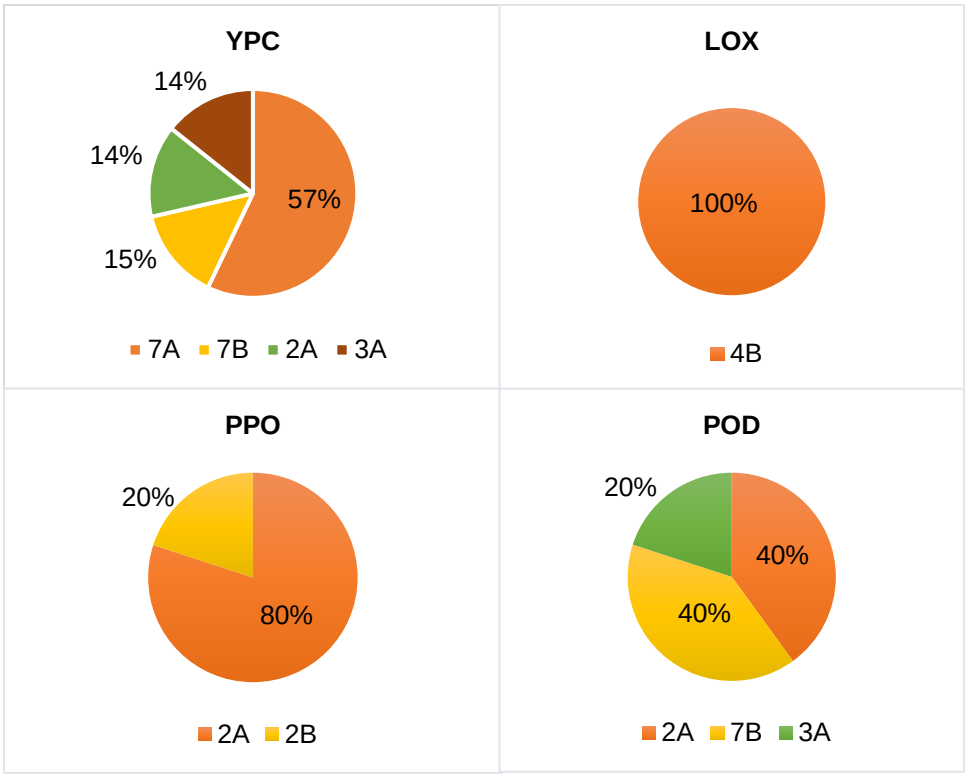

Figure 1.2. Effect of markers by chromosome for yellow pigment content (YPC), lipoxygenase (LOX), polyphenol oxidases (PPO), and peroxidase (POD).

In durum wheat, the color is derived from two principal pigments, carotenoids and anthocyanins. Carotenoids are responsible for the yellow color of the endosperm, whereas anthocyanins are responsible for the blue, red, and purple colors of durum grains (Ficco et al., 2014). In durum wheat, carotenoids are responsible for a wide range of nutrients. Carotenoids are tetraterpenoids (C40 chemicals), which are made up of isoprene units that are connected together in a linear and symmetrical fashion (Colasuonno et al., 2019). Dehydrogenation, hydrogenation, cyclization, and oxidation processes can alter the basic cyclic structure, while a complex system of double bonds causes the high chemical reactivity (Oliver and Palou, 2000). More than 600 carotenoids have been found in plants, with carotenes and xanthophylls being the most prevalent in durum wheat. The carotenoid family's color spectrum consists of a range of shades of red, orange, and yellow (Britton, 1995; Khoo et al., 2011). Durum wheat endosperm contains a high concentration of carotenoids, a primary yellow color source (Ficco et al., 2014).

Genetic variation has a more significant impact on the carotenoid component than environmental factors (Rharrabti et al., 2003b; Pozniak et al., 2007; Taghouti et al., 2010). As previously mentioned,

there are two types of carotenoids found in durum wheat, and they can be found in a variety of biological systems: carotenoids and xanthophylls (Hentschel et al., 2002). Carotenoids are a kind of hydrocarbon that includes carotenoids, like *α*-carotene, *β*-carotene, and lycopene. Xanthophylls are oxygenated carotenoid derivatives and contain combinations of *β*-cryptoxanthin, lutein, and zeaxanthin (Trocolli et al., 2000; Panfili et al., 2004; Ficco et al. 2014). According to Beleggia et al. (2011), the carotenoid content in dry durum is 6.2 ± 0.13 mg/kg of dry durum weight. The predominant site of *α*-carotene and *β*-carotene in the kernel is in the germ, where they are found in abundance. Quantitative inheritance combined with additive effect genes determine the carotenoid content. The principal QTL for carotenoid content is located on chromosome 7, while more minor QTL can be found in both the durum wheat genomes (A and B); (Colasuonno et al., 2019). The *Psy* gene, which codes for the enzyme phytoene synthase, is the most critical gene in the carotenoid pathway. This gene can also code for *PSY1* enzymes, which are necessary for the preservation of the endosperm's carotenoids (Gallagher et al., 2004; Li et al., 2008). In different wheat species, the *Psy* gene possesses more than 50 alleles at the *Psy-A1* and *Psy-B1* loci, resulting in a total of more than 50 alleles. Pozniak et al. (2007) discovered that *Psy-B1* and another QTL on chromosome 7B act together to increase the accumulation of carotenoids in the endosperm. According to He et al. (2008), they examined three distinct environments and found that two haplotypes of *Psy*-A1 on chromosome 7A explained 20-28% of the phenotypic diversity in YPC. Table (1.1) lists all of the durum wheat markers responsible for YPC (Ficco et al., 2014).

Continuous cascade reactions in the carotenoid biosynthesis are responsible for the yellow color in pasta. The first reaction in the carotenoid biosynthesis pathway is the reaction of two molecules of geranylgeranyl pyrophosphate (GGPP) to generate phytoene. This reaction is catalyzed by the enzyme phytoene synthase (PSY) (Figure 1.4). A sequence of cascade events follows, resulting in an increase in the amount of conjugated double bonds from three in phytoene to eleven in lycopene. These reactions are catalyzed by phytoene desaturase (*PDS*), zeta-carotene isomerase (*Z-ISO*), zeta-carotene desaturase (*ZDS*), and CRTIDO, respectively (Shimidzu et al., 1996). The third reaction is through the operation of lycopene cyclase, lycopene cyclization takes place at both ends of the molecule, resulting in the formation of *β*-carotene or *α*-carotene, respectively (*e-LCY*). The carotenoid hydroxylases are

responsible for the fourth reaction: the synthesis of xanthophylls lutein and zeaxanthin (Colasuonno et al., 2019).

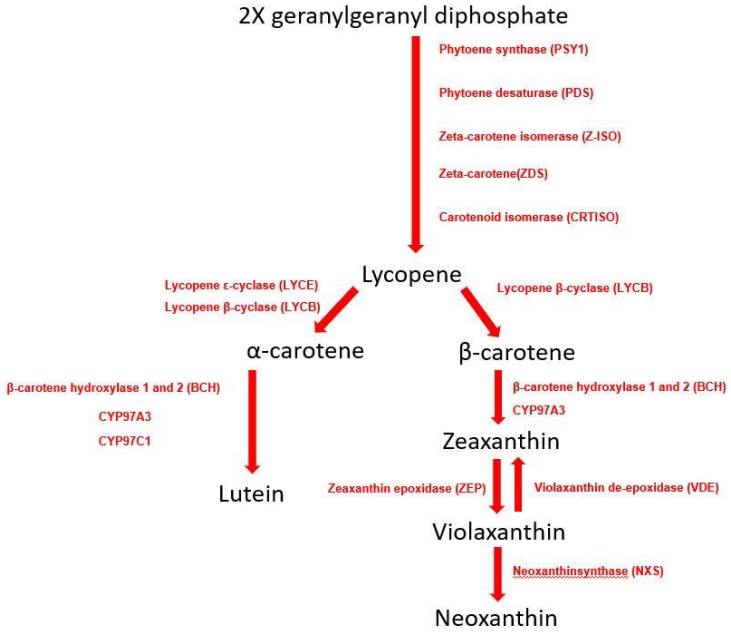

Figure 1.3. Carotenoid biosynthesis, main components are black, enzymes are red (adopted from Colasuonno et al.,2019).

Violaxanthin is then formed as a result of reversible double epoxidation of the rings in zeaxanthin, which is mediated by the enzyme zeaxanthin epoxidase. Violaxanthin is a precursor of abscisic acid. The pigments zeaxanthin, antheraxanthin, and violaxanthin (together referred to as the xanthophyll cycle pool) are involved in the dissipation of light energy in green plant tissues (Ficco et al., 2014). In this process, there are at least three rate-limiting events (Fig. 1.2): (i) the synthesis of phytoene, (ii) lycopene cyclization, and (iii) carotene hydroxylation, all of which occur early in the pathway. In light of the fact that the *PSY*-catalyzed reaction has been identified as the rate-limiting step of the wheat biosynthetic pathway, this reaction may play a vital role in controlling carotenoid accumulation in wheat (Lindgren et al., 2003; Cong et al., 2009; Colasuonno et al., 2019).

Table 1.1. Loci and markers responsible for yellow pigment content in durum wheat (adopted from Ficco et al., 2014).

Locus	Marker	Allele	Chromosome	Reference
Psy-A1	YP7A	*AsyA1a, PsyA1b*	7AL	He et al. 2008; Ravel et al. 2013
	YP7A-2	*psyA1a, PsyA1b, PsyA1c*	7A	He et al. 2009b; Ravel et al. 2013; Crawford et al. 2011
	Psy-A1_STS	*Psy-A1o*	7A	Singh et al. 2009
		Psy-A1e, Psy-A1p, Psy-A1a Psy-A1c, Psy-A1t,		
	Psy-A1_R_49	*Psy-A1a, Psy-A1k, Psy-A1ka, Psy-A1kb, Psy-A1e, Psy-A1ca, Psy-A1cb, Psy-A1p, Psy-A1ra, Psy-A1rb, Psy-A1j, Psy-A1t*	7A	Ravel et al. 2013
Psy-B1	YP7B-1	*Psy-B1a, Psy-B1b*	7BL	He et al. 2009a; Ravel et al. 2013
	YP7B-2	*Psy-B1c*		He et al. 2009b
Psy-B1	YP7B-3	*Psy-B1d*	7BL	He et al. 2009b
	YP7B-4	*Psy-B1e, Psy-B1a, Psy-B1aa, Psy-B1b, Psy-B1ca, Psy-B1m, Psy-B1d*		He et al. 2009b, Ravel et al. 2013
Zds-A1	YP2A-1	*Zds-A1a, Zds-A1b*	2AL	Dong et al. 2012
e-*LCY* (lutein)		*e-LYC3Aa, e-LYC3Ab*	3A	Crawford and Francki, 2013

Another class of pigments is anthocyanin pigment. These are the secondary plant products of flavonoid metabolism. They have long piqued the curiosity of biologists for their ability to protect plants from the environment. In terms of chemistry, anthocyanins are derived from anthocyanidin (aglycone), with sugar saccharide residues bonded at various hydroxylated locations on the basic structure of anthocyanins (Figure 1.4). Individual anthocyanins differ in the quantity of hydroxyl groups and sugars they contain, as well as the amount of aliphatic-aromatic acids connected to the sugars. They are also impacted by pH, temperature, the presence of a solvent, and the presence of co-pigments (Mazza, 2007). Anthocyanins are responsible for nearly all of the blue, purple, and red colors found in a wide variety of fruits, vegetables, and flowers (Delgado-Vargas et al., 2000; Winkel-Shirley, 2001). Many researchers have examined pigmented rice and maize, among other grains (Ryu et al., 1998; Salinas Moreno et al., 2005; Abdel-Aal et al., 2006; Del Pozo-Insfran et al., 2006; Sompong et al., 2011). The anthocyanins in bread wheat can be found in the grain as well as other parts of the plant such as the culm, coleoptile, anthers, and glumes (Khlestkina et al., 2010). Adom et al. (2005) found that the anthocyanins were concentrated in the outer layers of the kernel, where they are found in abundance in other antioxidant phytochemicals. Aleurone layers contain the blue wheat pigments, while pericarp layers contain the purple pigments (Zeven, 1991; Abdel-Aal and Hucl, 1999). Pigmented durum wheat, on the other hand, has yet to be thoroughly studied.

There are two major sections to the anthocyanin biosynthesis process shown in Figure (1.5): the general phenylpropanoid pathway and the series of reactions leading to flavonoid production. The first of these reactions involves converting phenylalanine to 4-coumaryl-CoA, with the help of phenylalanine ammonia lyase (PAL). In the flavonoid pathway, chalcone synthase catalyzes the 4-coumaroyl-CoA and 3-malonyl-CoA and produce chalcone. The enzyme chalcone isomerase then catalyzes the stereospecific isomerization of the yellow-colored chalcone to the colorless naringenin. Naringenin is transformed into dihydrokaempferol by the enzyme flavanone 3-hydroxylase. The process of changing dihydrokaempferol to anthocyanins uses different enzymes. At the beginning of the process, dihydrokaempferol changes to dihydroquercitin and after that must be reduced to leucocyanidins by the enzyme dihydroflavonol-4 reductase, which is followed by the formation of colored anthocyanins from the leucocyanidins (Ficco et al., 2014).

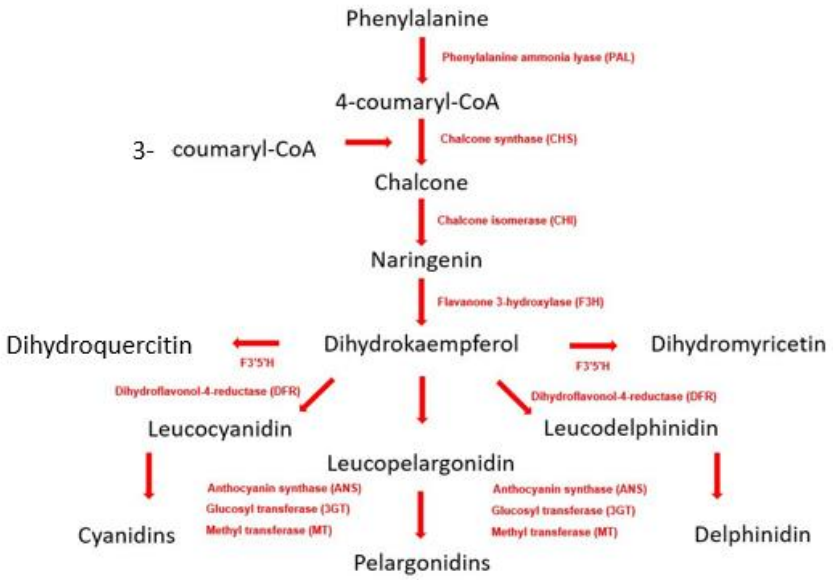

Figure 1.4. Anthocyanin biosynthesis, main components are black, enzymes are red (adopted from Ficco et al., 2014).

Further oxidation, dehydration, and glycosylation of the distinct leucoanthocyanidins might then result in the formation of the matching pigments: orange-red pelargonidin, red cyanidin, and blue delphinidin, amongst others. The variances in the sorts of glycosides and acyl groups attached depends on both the species and the diversity of the glycosides and acyl groups. According to the findings of Tereshchenko et al. (2013), the expression of the gene for flavanone 3-hydroxylase is the critical factor in the regulation of anthocyanin production in wheat. This finding is consistent with prior results in other plant species (Pelletier and Shirley, 1996). Very few researchers have examined the impact of environmental factors on wheat kernel anthocyanin expression. Based on the results of a study by Abdel-Aal and Hucl (2003), the growing season had more of an effect on a blue aleurone spring wheat line than it did on the anthocyanin content of commercial red and purple wheat cultivars. These results are most likely due to differences in pigment distribution inside the wheat kernels (Ficco et al., 2014).

Pasta color is determined by a balance between natural carotenoid yellow pigment buildup in the seeds and their oxidative degradation by enzyme activities, such as lipoxygenase (LOX), PPO, and POD (Carrera et al., 2007).

Enzymes Affect Pasta Color

Enzyme activity such as LOX, PPO, and POD have an impact on pasta color (Anderson and Morris, 2001; Kuzmanovic et al., 2020). Enzymes can be found in all parts of the kernel, including the

bran, germ, and endosperm. Enzymes in the semolina can be reduced with an ideal milling process (Rani et al., 2001; Borrelli et al., 2003). The majority of carotenoid pigments are reduced during milling, depending on the method used. The β-carotene concentration of semolina is reduced by 16.3% during pasta preparation, whereas milling reduces the β-carotene by 7.9%, (Aalami et al., 2007). According to Aalami et al. (2007), high levels of oxidative enzymes including LOX, PPO, and POD reduce β-carotene concentration in pasta products.

The yellow color of pasta is not necessarily dictated by the presence of a significant amount of YPC (Aalami et al., 2007). Lipoxygenase is the most important enzyme that determines color loss. When hydroperoxides are produced by catalyzing the deoxygenation of polyunsaturated fatty acids with 1,4-cis, cis pentadiene structures in the presence of 1,4-cis, cis pentadiene structures in the presence of 1,4-cis, cis pentadiene structures, the oxidation of carotenoid pigments and reduction of color in end-use pasta occurs (Siedow, 1991). In pasta products, the oxidative degradation caused by LOX during processing, as well as the processing parameters, has an impact on the yellowness of products (Borrelli et al., 2003; Aalami et al., 2007). Low LOX activity and a high pigment concentration in pasta products contribute to the yellow hue in the finished product. The amount of LOX present is more important in determining the yellow color of pasta than the amount of yellow pigment present (Aalami et al., 2007). Ficco et al. (2014) discovered that LOX's peroxidation of polyunsaturated fatty acids has an effect on carotenoid pigments (like β-carotene, xanthophylls, and chlorophylls) and results in the oxidative destruction of Γ-tocopherol. Due to its lower LOX activity when compared to the other wheat classes, durum wheat is preferred in making pasta with the high desirable yellow color. Aalami et al. (2007) found that the LOX content of seven Canadian durum wheat cultivars ranged between 10 and 16 lL O2/min/g, based on research conducted on the cultivars.

Lipoxygenase has an essential function in regulating the amount of yellow pigment produced during milling and processing. The amount of LOX in the durum can influence the color of the pasta. L-oxalate is subdivided into three types: LOX, LOX2, and LOX3. Lipoxygenase types are influenced by both genetics and environmental conditions (Troccoli et al., 2000). Lipoxygenase is an enzyme that belongs to the non-heme iron class of enzymes. Trono et al. (1999) and Pastore et al. (2000) found that three LOX isoforms in durum wheat have a negative correlation with the carotenoid content of semolina. Increased

levels of carotenoids and *Γ*-tocopherol can have a negative effect on the activity of LOX. However, after milling and removing the germ from the semolina, the amount of *Γ*-tocopherol in semolina is reduced. The negative association between YPC and LOX is responsible for controlling the number of carotenoids in the final product. Studies by Borrelli et al., (1999) and De Simone et al., (2010) have found that when LOX and YPC are low, only 4% of carotenoid loss occurs; on the other hand, when LOX and YPC are high, carotenoid loss increases to 20%.

Choosing pasta with a high YPC while also selecting against a high LOX activity results in pasta with high yellow color. The identification of genes that are associated with strong oxidation activity can assist breeders in improving the color of finished pasta products. The *LPX* is the gene that codes for the most significant LOX protein. Several alleles of this gene can be found on chromosomes four and five in wheat. The *Lpx-1* and *Lpx-2* are located on chromosome 4, where they regulate the expression of the LOX-1 and LOX-2 isoforms of this enzyme. The *Lpx-3* locus, which codes for the LOX-3 isoform, is located on chromosome 5 (Manna et al., 1998; Carrera et al., 2007; Zhang et al., 2008; Garbus et al., 2009; Chaonian et al., 2010; De Simone et al., 2010; Verlotta et al., 2010). The *Lpx-1* gene, which is located on the short arm of chromosome 4B, has a significant impact on the oxidation of yellow pigments during the pasta-making process. *Lpx-1* gene, Lpx-B1.1, Lpx-B1.2, and Lpx-B1.3, as well as three different Lpx-B1.1 allele: Lpx-B1.1a, Lpx-B1.1b, and the Lpx-B1.1c allele, have all been successfully mapped by several studies (Nachit et al., 2001; Hessler et al., 2002; Carrera et al., 2007; Zhang et al., 2008; Verlotta et al., 2010). According to Verlotta et al. (2010), the presence of Lpx-B1.1c in kernels results in reduced LOX activity.

Polyphenol oxidase and POD are two other enzymes that can help to minimize the amount of YI in pasta. Peroxidase enzyme is vital in the production of pasta with a brown index. The amount of POD activity present in kernels varies depending on the cultivar being used. Peroxidase is derived from a vast variety of chemicals and works by oxidizing hydrogen peroxide. The activity of this enzyme is weak when there is a deficiency of hydrogen peroxide during pasta processing (Icard-Vernière et al., 1999; Feillet et al., 2000; Fraignier et al., 2000). Several studies have been carried out to determine the activity of the POD enzyme, with varying results reported. A small QTL in the wheat genome affects the POD activity in the kernels. Chromosomes 1, 2, and 3 include a number of minor QTL, each of which is more active in a

distinct section of the durum wheat plant. *Per-1* is found in the coleoptile tissue whereas *Per-2* is found in the root tissue. *Per-3* is active in embryonic tissue, while *Per-4* is responsible for endosperm (found on chromosomes 4A, 7A, and 7D) (Asins and Perez de la Vega, 1985; Bosch et al., 1986; Liu et al., 1990; Ficco et al., 2014).

A group of enzymes known as PPO are responsible for the brown color of the finished pasta product. Two different types of reactions—*o*-diphenol oxidation and monophenol hydroxylation—are involved in PPO. The first is *o*-diphenol conversion to *o*-quinone, and the second is *o*-quinone conversion to monophenols (Anderson and Morris, 2001). A number of thiols, amines, and phenolic acids, such as sinapic acid and ferulic acid, can react with *o*-quinones to create melanin, which is responsible for the brown color of the end product. Polyphenol oxidases are responsible for the brown and dark colors found in plants such as tea, coffee, and dark raisins, among other things. When it comes to pasta, PPO activity has a detrimental impact on the bright yellow that is required for such products (Sissons, 2008). Presence of PPO in the aleurone layers of grain is a main factor in the darkening of the finished pasta product. Quinones are produced by PPO, resulting in the formation of brown polymers (Ficco et al., 2014). During the kneading process, as well as during hydration and drying, PPO activity increases (Dexter and Edwards, 1998). The most significant genes encoding the PPO enzyme in spring wheat may be found on chromosomes 2A and 2D. The *PPO-A2* and *PPO-A2* genes, which are located on chromosomes 2A and 2D, respectively, are responsible for a significant amount of phenotypic variation in wheat. *PPO-B2* gene has also been shown to be associated with other PPO genes and to have an effect on PPO activity. Because of the similarity of genomes in durum wheat and spring wheat (Durum wheat is tetraploid [AABB] and spring wheat is hexaploid [AABBDD]), this conclusion could be applied to both classes of wheat (Beecher and Skinner, 2011; Beecher et al., 2012).

Genome-wide Association Study (GWAS) vs. Bi-parental Mapping

During the past century, the majority of durum wheat breeding programs have improved yield potential and protein content (Clarke et al., 2010). In the 1970s, when International Maize and Wheat Improvement Center (CIMMYT) began releasing internationally tested cultivars, the durum improvement rate rose (Maccaferri et al., 2008). In the last two decades, as a result of market demand, breeders have begun to develop high-color lines. However, the time-consuming process of phenotyping for high yellow

color in pasta may have delayed the release of new improved cultivars. Because of the high cost of phenotyping at the present time, most breeders are forced to use genotype-based selection.

There is growing interest in breeding durum wheat using genomic techniques (Mengistu et al., 2016). With the help of molecular breeding techniques, new procedures such as AM (also known as GWAS), Bi-Parental mapping (also known as QTL mapping), and genomic selection (GS) are being developed to make it easier to develop and release superior cultivars with a high YI. Breeders have been employing MAS and GS to increase the YI (Pascual et al., 2020). Chromosome engineering is another method for obtaining desired characteristics in a short period of time, a goal that can be achieved through the transfer of an alien gene in to the durum genome. It is possible to transfer chromosome segments from Triticale to cultivated durum wheat (Gennaro et al., 2007). Kuzmanovic et al. (2020) reported that introducing the alien gene of the YPC characteristic related to the *Psy1* gene into a durum wheat cultivar could raise the YI up to 9% in cultivars such as Karur. They discovered that introducing alien genes to increase the YI in pasta had no negative consequences on the other traits in pasta products.

Bi-parental mapping has been used in most durum wheat quality trait research. Plant production, quality, and adaptability can be improved by employing linkage mapping of QTL using numerous markers linked with quantitative traits (Bernardo, 2016). Genetic and QTL mapping of plant disease resistance has typically been carried out using bi-parental mapping populations (Aoun et al., 2016).

Quality traits in wheat have been the subject of numerous bi-parental mapping investigations, which have yielded varying results. Several major QTL have been identified for TWT (Huang et al., 2006; Zhang et al., 2008; Patil et al., 2013), gluten strength (Patil et al., 2009; Kumar et al., 2013), and semolina color (Pozniak et al., 2007; Patil et al., 2008; Blanco et al., 2011; Fiedler et al., 2017).

On the other hand, AM is well-suited for the measurement and identification of quantitative character loci in family mapping and breeding populations, among other applications (Würschum, 2012). Association Mapping can test loci on elite lines for low heritability variables—like yield and yield components—and then analyze the results (Breseghello and Sorrells, 2006). In recent years, AM has been applied to plant studies, with many QTL being discovered and confirmed by this method (Agrama et al., 2007; Christopher et al., 2007; Casa et al., 2008; Ghavami et al., 2011). The primary goal of AM investigations is to identify associations between genotypes and phenotypic characteristics in a sample of

individuals based on the presence or absence of LD (Breseghello and Sorrells, 2006). The advantages of AM over QTL mapping in breeding populations include the use of large populations with phenotypic data collected over multiple locations and years, diverse genetic backgrounds with multiple allele polymorphisms, and the availability of populations and phenotypic data (Ghavami et al., 2011).

Agronomic variables such as grain yield, root architecture, plant height, and drought and salinity tolerance have all been studied using AM in durum wheat (Reimer et al., 2008; Maccaferri et al., 2010; Cane et al., 2014, Hu et al., 2015; Turki et al., 2015). This method has also been used to detect markers linked with plant disease resistance, such as resistance to Fusarium head-blight, Leaf-Rust Disease, and Stem-Rust Disease (Letta et al., 2014; Aoun et al., 2016).

Marker-assisted Selection in Durum Wheat

As molecular biology and high-throughput genotyping technology have improved, the field of molecular plant breeding has shifted away from phenotype-based selection toward genotype-based selection (Pascual et al., 2020). Molecular markers enable the scientific community to monitor, track, and exploit sequence variation in germplasm by discriminating between cultivars and breeding lines based on differences in DNA sequence. In functional genomics, structural genomics, and molecular breeding, several types of markers have been produced and are now critical components in breeding programs (Habash et al., 2009).

Due to recent advances in DNA markers like SNP, many genetic investigations have been simplified. According to a number of studies, molecular markers can be extremely effective tools for crop improvement by enhancing the efficacy of conventional plant breeding programs (Naraghi et al.,2019).

Currently, SNP are the most common type of molecular marker employed in assessing genetic diversity, population structure, familial kinship, and relationships in a wide range of plants. Because of the availability of spring wheat SNP markers, trait-marker association analysis in durum wheat may be performed with a high degree of efficiency (Hu et al., 2015). Because SNPs are widely dispersed and accessible to high multiplex detection technologies, they account for the majority of genetic variation across individuals (Ganal and Roder, 2007).

Marker-assisted selection is beneficial in cases where the molecular markers are related to enhancing quality attributes (Johnson et al., 2019). According to the USDA, early generation MAS can

provide breeders with an opportunity to choose the best lines and save substantial resources, as well as speed up the process of putting a new cultivar into the market (Johnson et al., 2019). Molecular MAS is an indirect selection strategy that uses molecular markers linked to the gene of interest to choose the best candidate (Ghavami et al., 2011). Marker-assisted selection is an excellent strategy that saves both money and time by eliminating the need for expensive and complicated phenotyping (Yousef and Juvik, 2001). Because of the use of whole-genome sequencing and genome-wide molecular markers, MAS has been able to accelerate the improvement of durum wheat genetic diversity (Vita and Taranto, 2019).

Both QTL and association mapping are methodologies that have been used to detect associations between durum wheat QTL traits, such as yellow pigment color and enzyme activity content. For example, Salsman et al. (2021) employed AM to identify the QTL for yellow pigment and grain color in more than 250 international durum wheat samples. Most breeding programs in the last decades have been directed to improve quality traits (Clarke et al., 2010). The recently released varieties showed that high yellow pigment color is a significant desirable trait in durum breeding programs (Digesù et al., 2009).

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CHAPTER 2. CHARACTERIZATION OF DURUM WHEAT LINES FOR COLOR-RELATED TRAITS IN PASTA

Abstract

Durum wheat with high yellow color is desirable for pasta production. The Yellow Pigment Content (YPC) in combination with the activity of enzymes such as peroxidases (POD) and polyphenol oxidases (PPO), can determine the final color of pasta. Therefore, one of the main objectives of a durum wheat breeding program is to produce durum wheat with high YPC and low enzyme activities. In order to find the best lines with high YPC, low soluble brown pigment (SBP), and enzymes activity, two recombinant inbred line (RIL) populations were developed and evaluated at two locations (Langdon and Williston, North Dakota) using a simple lattice design. Population one consisted of 196 RILs was developed from a cross between the durum cultivar 'Mountrail' with low PPO and YPC to 'Carpio' with high PPO and YPC. Population two had 196 RILs and was developed by crossing the durum cultivar 'Joppa' (High YPC and low PPO) to durum wheat experimental line D121118 (Low YPC and PPO). Phenotypic data was collected for PPO, POD, YPC, and soluble brown pigment (SBP); also, agronomy traits like yield and test weight (TWT) were measured to find lines with both high yield and good quality. In both populations several lines had higher yield, test weight, and YPC than the higher check cultivar (ND-Riveland). Also, in both populations, several lines were lower in SBP, PPO, and POD enzyme activity than all parents and checks.

Introduction

The most widely farmed and economically significant cultivar of tetraploid wheat is durum (*Triticum turgidum* ssp. durum Desf.), which is well-suited to semi-arid regions, especially in Mediterranean countries and North America. While most of the world's durum wheat is cultivated in Italy and Canada (followed by Syria and the United States), it is also grown in Algeria, Morocco, Russia, Turkey, and Tunisia (Hu et al., 2015).

Given that high yield is essential for commercially valuable varieties (Trono et al., 1999), quality factors have also been a prominent consideration in the selection of the best candidate(s) for grain marketing among the durum wheat lines. Durum wheat quality is a factor that is always changing in response to market needs and consumer preferences for certain end-product characteristics (Troccoli et

al., 2000). The preservation of a fine texture, the durability of yellowness after cooking, and the sensory appeal of pasta are all desired pasta quality characteristics (Alzuwaid et al., 2021).

Color is one of the most important aspects influencing the quality of durum wheat and the purchasing decision of end products by the consumer (Borrelli et al., 2008). The presence of a rich yellow color in the finished pasta product is an important quality factor for the durum wheat market (Troccoli et al., 2000). The carotenoid pigments and the enzymatic activity responsible for their oxidative breakdown are primarily responsible for the bright yellow color of final products (Borrelli et al., 2008). Additionally, carotenoids, which are naturally occurring antioxidant molecules, are extremely significant in the nutritional and health aspects of foods, as well as in the preservation of their freshness and shelf life (Shahidi, 2000).

In a study by Aalami et al. (2007b), it was shown that different cultivars of durum wheat have varying YPC. They identified two lines (PDW-215 and WH-896) with a high YPC of 5 and 5.15 ppm, respectively. In another study (Aalami et al., 2007a), the cultivar DWR-2006 was shown to have the highest YPC (6.35 ppm), while the cultivar MACS-2694 had the lowest amount of YPC (3.12 ppm). Yellow pigment content can be related to TWT (Wang and Fu, 2020). In their study, they found with decreasing kernel size, YPC increased significantly in durum cultivars that were tested. They also found that kernel size could influence pasta color, despite the fact that small kernels contained significantly more yellow pigment than large kernels, pasta made from small kernels was slightly darker, redder, and had less yellow color. These results were most likely due to the higher levels of semolina ash and protein in small kernels, which had a negative impact on pasta color (Wang and Fu, 2020). Furthermore, about 8% of the carotenoid pigments are lost during milling, and another 16% are lost during pasta processing (Borrelli et al., 1999). The color of dry and cooked pasta is the result of a delicate balance between the buildup of natural YPC in the seeds and the actions of kernel enzymes. Pasta preparation results in a significant loss of yellow pigments, and this loss is largely attributable to enzyme activities (Carrera et al., 2007).

Three important enzymes that can affect pasta color are lipoxygenases (LOX), PPO, and POD. For pasta preparations, products with lower LOX activities and higher yellow pigment content are preferable, especially when it comes to the color of the finished product (Aalami et al., 2007b). Lipoxygenase activity varies greatly across durum wheat lines; it is a hereditary trait that is also affected

by the environment. The amount of LOX varies among durum wheat varieties. Aalami et al. (2007b) reported the highest amount of LOX activity was 6.9 (U/g) while the lowest number was 1.4 (U/g) in the durum lines they tested.

In addition to the effect of enzyme activities on YPC, brown pigment can be influenced by enzyme activities. Pasta browning can be caused by either enzymatic or non-enzymatic processes (Matsuo and Irvine, 1967; Kobrehel et al., 1974; Cabas-Lühman, 2019). Brown-cupric soluble protein and/or Maillard reaction products can result in non-enzymatic brownness (Matsuo and Irvine, 1967). The activity of PPO has a substantial impact on the color and brownness of pasta (Yoruk and Marshall, 2003). Kobrehel et al. (1974) found that PPO and POD are among the enzymes responsible for the formation of brown pigment. Polyphenol oxidases are enzymes that perform a variety of functions, such as catalyzing the hydroxylation of o-monophenols to o-diphenols, among others. Additionally, PPO has the ability to oxidize o-dihydroxyphenols to o-quinones (Van Gelder et al., 1997). Polyphenol oxidases are virtually eliminated throughout the milling process (Baik et al., 1994). However, if a small amount of PPO persists, it can lead to major issues with the color and quality of semolina and the final pasta products (Demeke et al., 2001; Rani et al., 2001; Verlotta et al., 2010). In durum wheat, PPO activity ranges from 0.04 to 0.2 (ΔA475/min g) (Cabas-Lühman, 2019). On the other hand, POD catalyzes the oxidation of a large amount of phenols and aromatic compounds using hydrogen peroxide (H2O2) as a substrate, resulting in a brown color (Gaspar et al., 1982). Pasta products made from cultivars with high POD activity were browner than those made from low POD activity (Kobrehel et al., 1974; Taha and Sagi, 1987).

For durum wheat, yellow carotenoid pigment concentration is an important quality parameter determined by both pigment buildup and pigment breakdown through enzyme activities. Thus, durum wheat breeding efforts around the world focus on assessing a wide variety of germplasm that could be used to increase grain yield and quality of durum lines that could be suitable for commercial applications. In order to achieve these goals, the objective of this study was: to identify durum lines with high yield potential, high YPC, low SBP, and low PPO and POD enzymes activities.

Material and Methods

Plant Material

We developed two RIL mapping populations. Population one (POP1) was developed from crossing the high YPC and low PPO activity durum cultivar “Joppa” (Elias and Manthey, 2016) with the low YPC and low PPO activity experimental line D121118. Joppa was chosen because of its high grain yield potential, disease resistance, and excellent end-use attributes. D121118 a high-quality experimental line was developed in the durum wheat-breeding program at North Dakota State University (NDSU).

The second RIL population (POP2) was developed by crossing the durum cultivar "Mountrail" (Elias and Miller, 2000), which has low YPC and low PPO activity, with the durum cultivar "Carpio" (Elias et al., 2015), which has high YPC and high PPO activity. Carpio was released because of its high yield potential, good quality, and disease resistance. Carpio was developed from a cross between two durum experiment lines (D95580 and D955595). Elias and Miller (2000) released Mountrail in 2000 because of its excellent yield potential and good quality. Mountrail was developed by crossing the durum experimental line D8479 with the durum cultivar Renville.

The single seed descent (SSD) method was used to develop both RIL populations in this study. The original crosses were made in the Lord and Burnham greenhouse at NDSU over the winter of 2016. In the spring greenhouse of 2017, F_1 seeds were planted; F_2 seeds were harvested and advanced as SSD to generate the subsequent generations. To shorten the duration and increase the number of seeds, one spike per plant was picked and planted as $F_{4:5}$ head rows in the 2019 winter nursery in New Zealand. $F_{4:6}$ lines were harvested as bulk and planted in two replicates at two locations (Langdon and Williston, North Dakota [ND]). Langdon is located in northeastern ND, while Williston is located in northwestern ND.

A total of 196 RIL were used in each population, including parents and checks. We used two-row plots in Langdon and five-row plots in Williston to evaluate all traits in both populations. The experimental design was a simple lattice design for both populations. Divide (Elias and Manthey, 2007), and ND-Riveland (Elias and Manthey, 2019), were used as checks because they are the predominantly grown cultivars in ND. Divide was chosen due to its high-yielding and high-quality characteristics (Elias and Manthey, 2007). ND-Riveland was chosen because of its high yield potential and outstanding end-use qualities (Elias and Manthey, 2019). All parents were also included in the tests.

For breeding purposes, data on Fusarium Head Blight resistance, leaf disease, plant height, and lodging were recorded in addition to yield, test weight and color phenotyping traits.

Phenotyping

Yield and Test Weight

Durum wheat plots were harvested with a combine in early September of 2019. Following harvest, the samples were dried in an oven (for 72 hours at 32 C) and then cleaned. When the moisture content reached 12 percent by weight, both the seed yield and the TWT of the plots were measured. Test weight was determined using AACC method 55-10.01 (2010).

Yellow Pigment Content

A modified AACCI approved method 14-50.01 was used to determine the yellow pigment content where 4 Instead of 8 g of whole wheat flour we used. The solvent used in a 5:1 ratio by mixing 20 mL of water saturated n-butanol reagent (WSB) with four grams of flour. After shaking the mixture for two minutes on a vortex mixer and letting it rest for one hour, the samples were centrifuged for five minutes at 18,514 x *g* relative centrifuge force (RCF) (Eppendorf 5810R centrifuge, Rotor: F-34-6-38, Radius: 11.5cm). A spectrophotometer (VWR UV-1600 PC) was used to measure the absorbance of the supernatant at 436 nm. Using the *β*-carotene extinction coefficient 1.6632, measurements per extracted sample were converted to yellow pigment concentration (µg/g or ppm).

Soluble Brown Pigment Content

We modified the method used by Matsuo and Irvine (1967) to determine the soluble brown pigment content. Four ml deionized distilled water was mixed with two grams of whole wheat flour. The mixture was mixed twice on a vortex mixer, then allowed to rest for five minutes before being centrifuged for six minutes at 18,514 x *g* relative centrifuge force (Eppendorf 5810R centrifuge, Rotor: F-34-6-38, Radius: 11.5cm) (RCF). The supernatant (1.5 ml) was centrifuged for 10 minutes in an Eppendorf tube. A spectrophotometer (VWR UV-1600 PC Spectrophotometer) was used to measure the absorbance of the supernatant at 400 nm. OD_{400} was recorded as the final measurement.

Polyphenol Oxidase Enzyme Activity

In this study, the activity of PPO was measured using the AACCI-approved method 25-85.01. Five mm of L-3, 4 dihydroxyphenylalanine (L-DOPA) in 50 mm of 3-(N-morpholino) propanesulfonic acid

(MOPS) buffer pH 6.5, 0.02% solution Tween-20 (1.5 ml solution) was added to the ground material in a micro-centrifuge tube (200 mg, whole wheat flour). In order to start the reaction properly, the tubes were placed on an orbital shaker (Glas-Col, Terre Haute, IN, USA) and rotated for one hour at room temperature. The samples were then centrifuged at 14,000 x *g* for two minutes. At 475 nm, the absorbance of the supernatant was measured using a VWR UV-1600 PC Spectrophotometer. Each sample had a control sample that was treated with a solution that did not include L-DOPA. Each sample's absorbance was subtracted from the control's absorbance. The enzyme activity was measured in ΔA475/ min gr of material, with a change in absorbance unit per minute regarded as one unit of enzyme activity.

Peroxidase Enzyme Activity

The technique published by Honold and Stahmann (1968) and Fraignier et al. (2000), with minor changes, was used to measure peroxidase activity. Whole wheat flour (150 mg) was mixed in 1.5 ml of 50 mm phosphate citrate buffer pH 4.6 (1:10 w/v) and shaken in an orbital shaker (Glas-Col, Terre Haute, IN, USA) for two hours at room temperature. After finishing the rotation, samples were centrifuged at 14,000 x for 10 minutes. The supernatant (150µl) was then combined with 1.5 ml of a solution containing five mm guaiacol, 10 mm H_2O_2, and 50 mm phosphate citrate buffer (pH 5). The oxidation of guaiacol to tetrahydroguaiacol in the presence of H_2O_2 was measured spectrophotometrically (VWR UV-1600 PC Spectrophotometer) at 470 nm for a minute, recording every 15 seconds to calculate POD enzyme activity. The total activity was measured in units per gram of dry mass (U/g). A change in absorbance unit per minute was regarded as one unit of enzyme activity.

Statistical Analysis

At the field experiment, two RIL populations were planted in both locations using a simple lattice design. Each location was considered as a separate environment (two environments total), each with two populations. Environments were treated as random effects, whereas genotypes were treated as fixed effects. When the ratio (Fmax) of the effective error variances for each trait was less than 10-fold, the environment was declared homogenous (Tabachnik and Fidell, 2001). When error variances were homogeneous, a combined analysis across locations was conducted. Statistical Analysis System (SAS) version 9.4 for Windows (SAS Institute Inc., Cary, NC, USA) was used to analyze the data. LSMeans

were computed, and mean separation was conducted with a macro statement at a 95% level of confidence (F tests: $P<0.05$).

Results

Error variance from single location analysis were checked for homogeneity before combining data across locations. Single location error variances for both populations were homogeneous for all traits and therefore data were combined and means across locations were reported and discussed. In general, breeding programs select and advance the top 5% to 10% of lines for possible release as cultivars. In this study, the top five lines across both locations for each trait were selected and compared to the checks. Single location means were reported only for observation purposes. A correlation analyses among the traits were conducted. There were no significant correlation or association among the traits with the exception of the correlation between yield and TWT, which was 28%.

Yield

The primary goal of durum wheat breeding projects around the world has been to develop durum wheat cultivars that provide a high grain yield and good quality. The Langdon location had higher yield (3,927 kg/ha) than the Williston location (3,371 kg/ha) for POP1 (Table 2.1). Similarly, POP2 yield in Langdon (3,930kg/ha) was higher than Williston (3,491 kg/ha) (Table 2.2). This was expected since the Williston location is characterized by dryer environmental conditions.

There were significant differences among genotypes in both populations. Several progenies in the two populations had higher yield than the high yielding check ND-Riveland. In POP1 at least five experimental lines had higher yield than ND-Riveland (Table 2.1). In POP2, only the two experimental lines E162 and E187 had higher yield than ND-Riveland (Table 2.2). There is a potential for developing high yielding cultivars from both populations since several lines out yielded the checks across locations.

Test Weight

The soundness of grains can be determined by their TWT. When it comes to durum wheat, at the same amount of TWT, there is a negative relationship between kernel size and yield of flour and semolina (Wang and Fu, 2020). In general, smaller kernels, provide lower semolina yield than bigger kernels. Grain grading uses test weight as a predictor for flour yield, particularly for wheat. Williston showed slightly

higher TWT than Langdon in both populations. In general, Williston had smaller kernel sizes than the Langdon location. In POP2, both parents had high TWT (Carpio 81.1 Kg/hL and Mountrail 79.5 Kg/hL).

In both populations and across locations, all checks had lower TWT than the top five experimental lines (Table 2.1 and 2.2). There is a potential of developing high-test weight cultivars from both populations since several lines had higher test weight than the check cultivars.

Table 2.1. Mean grain yield and test weight of top five lines in population 1 (Joppa X D121118) at Langdon, Williston and across locations grown in 2019.

Genotype	Yield (kg/ha)	Genotype	TWT (kg/hL)
---------- Across both locations----------			
E190	4226	E126	81.9
E148	4200	E156	81.7
E108	4184	E122	81.7
E128	4157	E173	81.6
E37	4142	E62	81.4
Joppa	3907	Joppa	80.4
D121118	3592	D121118	78.9
Mountrail	3650	Mountrail	78.7
Divide	3579	Divide	80.1
ND-Riveland	3717	ND-Riveland	79.2
Mean	3649		80.0
Maximum	4226		81.9
Minimum	3048		77.5
LSD	195		0.5
CV%	6.6		1.0
----------Langdon----------			
E190	4865	E126	82.2
E57	4816	E167	81.4
E65	4768	E170	81.1
E33	4677	E168	81.0
E11	4657	E105	80.8
Joppa	4276	Joppa	76.5
D121118	3701	D121118	77.1
Mountrail	3621	Mountrail	76.5
Divide	3636	Divide	78.9
ND-Riveland	3718	ND-Riveland	78.6
Mean	3927		79.0
Maximum	4865		83.1
Minimum	2981		73.0
LSD	N.S		0.8
CV%	8.5		2.1
----------Williston----------			
E108	4180	E122	83.7
E112	4136	E70	83.5
E100	4090	E73	83.3
E89	4054	E156	83.2
E113	4023	E100	83.0
Joppa	3524	Joppa	81.1
D121118	3500	D121118	80.8
Mountrail	3684	Mountrail	81.4
Divide	3511	Divide	81.4
ND-Riveland	3764	ND-Riveland	79.9
Mean	3371		81.0
Maximum	4180		83.7
Minimum	2509		77.9
LSD	199		0.5
CV%	9.4		1.3

Table 2.2. Mean grain yield and test weight of top five lines in population 2 (Mountrail X Carpio) at Langdon, Williston and across locations grown in 2019.

Genotype	Yield (kg/ha)	Genotype	TWT (kg/hL)
----------Across both locations----------			
E162	4506	E55	82.5
E187	4355	E105	82.4
E88	4326	E134	82.0
E46	4228	E182	82.0
E52	4228	E19	82.0
Mountrail	3776	Mountrail	79.5
Carpio	3719	Carpio	81.1
Divide	3680	Divide	80.7
Joppa	3993	Joppa	80.9
ND-Riveland	4164	ND-Riveland	80.6
Mean	3714		80.1
Maximum	4506		82.5
Minimum	3118		76.3
LSD	188		0.53
CV%	6.2		1.35
----------Langdon----------			
E162	5635	E145	82.6
E187	4808	E55	82.6
E135	4718	E105	82.1
E88	4708	E158	82.1
E174	4669	E134	82.0
Mountrail	4001	Mountrail	79.6
Carpio	3819	Carpio	79.7
Divide	4180	Divide	80.2
Joppa	4543	Joppa	80.5
ND-Riveland	4360	ND-Riveland	79.9
Mean	3930		79.6
Maximum	5365		82.6
Minimum	3004		75.1
LSD	299		0.8
CV%	8.6		1.6
----------Williston----------			
E46	4311	E188	83.3
E19	4128	E19	83.3
E41	4122	E164	83.1
E43	4082	E182	82.9
E89	4058	E178	82.8
Mountrail	3544	Mountrail	79.5
Carpio	3640	Carpio	82.4
Divide	3187	Divide	81.1
Joppa	3427	Joppa	81.2
ND-Riveland	3985	ND-Riveland	81.3
Mean	3491		80.7
Maximum	4311		83.3
Minimum	2809		76.2
LSD	223		0.7
CV%	8.4		1.5

Yellow Pigment Content

Pasta's bright yellow color is a popular feature among consumers. The ratio of yellow to brown pigment content has influence on the degree of yellowness in pasta. The amount of yellow pigment in durum wheat semolina has a significant impact on the final pasta products (Borrelli et al., 2008).

Comparing both locations, Williston trials had slightly higher YPC than Langdon. In POP1, the parent Joppa had significantly higher YPC than the parent D121118 (Table 2.3). Entries E78, E49, and E15 had higher YPC than all the checks with the exception of the cultivar Joppa. Among entry lines, E78 had the highest YPC at both locations. In POP2, the parent Carpio had significantly higher YPC than the parent Mountrail (Table 2.4). All top five experimental lines had higher YPC than all the checks and parents. In POP2, the entry E1 and E101 had high YPC at both locations, Langdon and Williston, ND. The three entries E78 from POP1 and E1 and E101 from POP2 have stable YPC at both locations.

In a study conducted by Cabas-Lühmann (2019) the range of YPC was between 6.7 to 8.8 ppm in semolina. In our study, the entry lines 1, 82, and 101 in POP2 had higher than 10 ppm YPC. In Williston, ND the two lines E1 (11.33 ppm) and E101 (11.31 ppm) had very high YPC (Table 2.4). Entry lines 1, 82, and 101 would be good parents in a crossing block for developing high YPC durum cultivars.

Soluble Brown Pigment

Pasta color is a critical parameter for pasta quality, therefore, critical to consumers. There are three aspects to pasta color: desired yellow, less-desirable brown, and red color (depending on the drying conditions) (Kobrehel et al., 1974). Pasta browning has different biological causes (Feillet et al., 2000). Enzyme and non-enzyme activities have an important effect on SBP (Kobrehel et al, 1974). In total, drier locations such as Williston had more SBP than more humid locations such as Langdon. Previous research conducted by Cabas-Lühmann (2019) showed similar results.

Table 2.3. Yellow pigment content, soluble brown pigment, polyphenol oxidase, and peroxide activity of top five entry lines in population 1 (Joppa X D121118) at Langdon, Williston and across locations grown in 2019.

Genotype	YPC (ppm)	Genotype	SBP (OD_{400})	Genotype	PPO (ΔA475/min)	Genotype	POD (U/g)
----------Across both locations----------							
E78	8.73	E32	0.000	E188	0.048	E51	2.39
E49	8.35	E78	0.000	E128	0.066	E176	2.43
E15	8.28	E184	0.000	E36	0.069	E169	2.52
E186	8.12	E24	0.095	E164	0.078	E60	2.62
E155	8.07	E117	0.109	E92	0.079	E117	2.66
Joppa	8.72	Joppa	0.127	Joppa	0.255	Joppa	4.11
D121118	6.70	D121118	0.168	D121118	0.280	D121118	6.10
Mountrail	5.62	Mountrail	0.132	Mountrail	0.048	Mountrail	6.18
Divide	6.95	Divide	0.116	Divide	0.306	Divide	3.54
ND-Riveland	8.08	ND-Riveland	0.154	ND-Riveland	1.234	ND-Riveland	5.16
Mean	7.25		0.157		0.269		5.57
Maximum	8.73		0.270		1.234		16.03
Minimum	5.62		0.000		0.048		2.39
LSD	0.10		0.016		0.068		0.01
CV%	6.8		23.1		53.7		52.4
----------Langdon----------							
E78	8.89	E32	0.000	E20	0.091	E95	0.03
E7	8.51	E78	0.000	E84	0.099	E60	0.04
E189	8.44	E184	0.000	E123	0.099	E169	0.07
E49	8.43	E24	0.068	E43	0.100	E48	0.11
E186	8.40	E66	0.096	E50	0.101	E93	0.14
Joppa	8.67	Joppa	0.110	Joppa	0.145	Joppa	3.85
D121118	7.07	D121118	0.179	D121118	0.110	D121118	6.93
Mountrail	5.95	Mountrail	0.139	Mountrail	0.120	Mountrail	8.27
Divide	7.13	Divide	0.112	Divide	0.147	Divide	2.24
ND-Riveland	8.06	ND-Riveland	0.137	ND-Riveland	0.301	ND-Riveland	4.22
Mean	7.39		0.145		0.135		5.71
Maximum	8.89		0.374		0.3		26.81
Minimum	5.95		0.000		0.09		0.03
LSD	0.17		0.020		0.11		0.02
CV%	6.9		31.9		16.9		103.9

Table 2.3. Yellow pigment content, soluble brown pigment, polyphenol oxidase, and peroxide activity of top five entry lines in population 1 (Joppa X D121118) at Langdon, Williston and across locations grown in 2019 (Continued).

Genotype	YPC (ppm)	Genotype	SBP (OD_{400})	Genotype	PPO (ΔA475/min)	Genotype	POD (U/g)
----------Williston----------							
E78	8.57	E32	0.000	E36	0.000	E73	2.38
E49	8.28	E78	0.000	E128	0.000	E74	2.92
E15	8.25	E184	0.000	E188	0.000	E70	2.94
E134	8.15	E154	0.117	E101	0.030	E75	3.34
E113	8.06	E148	0.120	E164	0.036	E174	3.98
Joppa	8.78	Joppa	0.142	Joppa	0.036	Joppa	4.38
D121118	6.32	D121118	0.152	D121118	0.451	D121118	5.24
Mountrail	5.29	Mountrail	0.123	Mountrail	0.000	Mountrail	4.12
Divide	6.78	Divide	0.121	Divide	0.466	Divide	4.83
ND-Riveland	8.10	ND-Riveland	0.172	ND-Riveland	2.164	ND-Riveland	6.14
Mean	7.12		0.168		0.400		5.42
Maximum	8.77		0.313		2.164		7.60
Minimum	5.29		0.000		0.000		2.38
LSD	0.11		0.024		0.136		0.02
CV%	7.4		24.9		69.5		14.1

Table 2.4. Yellow pigment content, soluble brown pigment, polyphenol oxidase, and peroxide activity of top five entry lines in population 2 (Mountrail X Carpio) at Langdon, Williston and across locations grown in 2019.

Genotype	YPC (ppm)	Genotype	SBP (OD_{400})	Genotype	PPO (ΔA475/min)	Genotype	POD (U/g)
----------Across both locations----------							
E101	10.14	E28	0.363	E153	0.099	E38	2.47
E1	10.13	E176	0.367	E27	0.102	E102	2.81
E82	10.01	E145	0.369	E58	0.105	E45	2.92
E67	9.86	E16	0.370	E100	0.105	E116	2.93
E61	9.85	E118	0.374	E24	0.107	E94	3.02
Mountrail	5.92	Mountrail	0.421	Mountrail	0.133	Mountrail	20.22
Carpio	9.10	Carpio	0.499	Carpio	0.353	Carpio	28.15
Divide	7.62	Divide	0.431	Divide	0.143	Divide	23.62
Joppa	9.26	Joppa	0.668	Joppa	0.176	Joppa	29.67
ND-Riveland	8.64	ND-Riveland	0.477	ND-Riveland	0.341	ND-Riveland	24.82
Mean	7.73		0.430		0.231		7.00
Maximum	10.17		0.668		0.407		33.35
Minimum	5.19		0.363		0.099		2.47
LSD	0.18		0.029		0.014		1.25
CV%	13.6		9.8		39.2		66.9
----------Langdon----------							
E174	9.08	E27	0.314	E123	0.115	E94	0.00
E101	9.02	E6	0.316	E52	0.115	E100	0.00
E1	8.95	E5	0.318	E112	0.117	E161	0.00
E82	8.92	E28	0.318	E164	0.117	E184	0.00
E117	8.76	E48	0.319	E80	0.118	E179	0.09
Mountrail	5.72	Mountrail	0.380	Mountrail	0.127	Mountrail	33.75
Carpio	8.14	Carpio	0.510	Carpio	0.330	Carpio	47.07
Divide	7.29	Divide	0.385	Divide	0.142	Divide	39.35
Joppa	8.71	Joppa	0.382	Joppa	0.133	Joppa	49.70
ND-Riveland	8.53	ND-Riveland	0.417	ND-Riveland	0.282	ND-Riveland	41.36
Mean	7.08		0.365		0.248		5.90
Maximum	9.08		0.510		0.431		55.69
Minimum	3.79		0.314		0.115		0.00
LSD	0.30		0.014		0.020		2.46
CV%	13.2		6.4		38.2		151.4

Table 2.4. Yellow pigment content, soluble brown pigment, polyphenol oxidase, and peroxide activity of top five entry lines in population 2 (Mountrail X Carpio) at Langdon, Williston and across locations grown in 2019 (Continued).

Genotype	YPC (ppm)	Genotype	SBP (OD_{400})	Genotype	PPO (ΔA475/min)	Genotype	POD (U/g)
----------Williston----------							
E1	11.33	E176	0.338	E153	0.064	E25	4.87
E101	11.31	E16	0.392	E58	0.073	E102	4.89
E67	11.16	E179	0.395	E24	0.075	E38	4.89
E61	11.11	E149	0.398	E39	0.076	E112	5.29
E82	11.06	E184	0.399	E182	0.078	E113	5.39
Mountrail	6.13	Mountrail	0.462	Mountrail	0.137	Mountrail	6.86
Carpio	10.09	Carpio	0.488	Carpio	0.375	Carpio	8.97
Divide	7.79	Divide	0.477	Divide	0.146	Divide	8.10
Joppa	9.79	Joppa	0.954	Joppa	0.218	Joppa	9.49
ND-Riveland	8.76	ND-Riveland	0.537	ND-Riveland	0.399	ND-Riveland	8.26
Mean	8.38		0.495		0.214		8.11
Maximum	11.33		0.954		0.454		17.93
Minimum	5.57		0.388		0.064		4.87
LSD	0.18		0.057		0.020		0.50
CV%	14.6		15.2		43.4		21.8

Joppa had lower SBP than experiment line D121118 (0.127 and 0.168 OD_{400}, respectively). The top five lowest SBP lines across both locations had a lower amount of SBP than all checks, including parents (Table 2.3). The best entries were E32, E78, and E184 (zero OD_{400}). The YPC and SBP results confirm that entry line E78 (with highest YPC [8.74 ppm] and lowest SBP [zero OD_{400}]) could be a good parent for developing high quality durum cultivars because of its high YPC and low SBP. Based to our knowledge through the literature, this is the first time a line with high YPC and low SBP has been reported. Also, the other two entries (E32 and E184) have high YPC (7.59 and 7.68 ppm, respectively). In both locations, the range was 0.304 with the maximum amount of SBP (0.668 [OD_{400}]) and minimum SBP (0.363 [OD_{400}]). SPB of Carpio was not significantly different that Mountrail (Table 2.4). In POP2, the five entry lines with the lowest SBP had lower SBP than all the checks and parents. Entry 28 (0.363 [OD_{400}]) and E176 (0.367 [OD_{400}]) had the lowest SBP across locations.

Polyphenol Oxidase Enzyme Activity

The enzymes expressed in durum wheat are critical because they have an impact on the final quality of the end-used pasta (Aalami et al., 2007a). One of the main enzymes which affects the color of pasta is PPO. In this study, two populations were used to find the best lines with the lowest amount of PPO. Carpio was selected as parent for POP2 because of its high PPO while Joppa, Mountrail, and D121118 were selected because of their low PPO. In our study, Carpio had high PPO as expected while only Mountrail had low PPO (Tables 2.3 and 2.4). The very high PPO of D121118 in Williston may have resulted in its high PPO across locations (Table 2.3). In POP1, ND-Riveland, which was used as a check, had the highest amount of PPO (1.234 [(ΔA_{475}/min g]) while Mountrail (0.048 [ΔA_{475}/min g]) had the lowest PPO enzyme activity (Tables 2.3 and 2.4). Cabas-Lühmann (2019) reported similar results for ND Riveland and Mountrail. She also reported a PPO range of 0.04 to 0.20 (ΔA_{475}/min g), while our range for entry lines was between 0.049 and 0.640 (ΔA_{475}/min g). In POP1, all top five experimental lines had lower PPO than the checks with the exception of Mountrail (Table 2.3). In POP2, Carpio and ND-Riveland were the lines with high PPO activity, while Mountrail as one of the parents in this population had the lowest PPO (Table 2.4). In POP2, all top five experimental lines had lower PPO than all checks including parents (Table 2.4). The best entry for low PPO was the line E153 with 0.099 (ΔA_{475}/min g).

Peroxidase Enzyme Activity

In durum wheat, POD is regarded as detrimental since it can cause changes in the grain's color, flavor, and nutritional value (Burnette, 1977). Pasta manufactured from durum cultivars with high POD activity had a brownish color and the brown index of pasta has a positive link with POD activity (Kobrehel et al., 1974). The effects of POD can be controlled by genotype and/or the environment. In this study, two populations were tested for the amount of POD to find the relationship between enzyme activities and other color-related traits in durum wheat. In POP1, Joppa had significantly lower POD than the parent D121118. All top five experimental lines had lower POD than the low POD parent, Joppa as well as all the checks. The lowest POD was reported for E51 (Table 2.3). In POP2, the parent Mountrail had significantly lower POD than the parent Carpio. All top five experimental lines had lower POD than both parents and the checks (Table 2.4). The checks had the highest amount of POD, with an activity range between 20.22 to 29.67 (U/g) in across location (Table 2.4). Cabas-Lühmann (2019) showed similar ranges of POD activity (33.7 to 37.2 [U/g]) in durum wheat. Several lines in both populations had lower POD that can be used in breeding programs to develop durum cultivars with low POD.

Discussion

The choice of parental lines significantly impacts the power and accuracy of QTL detection (Jansen et al., 2001). In other words, allelic polymorphism and phenotypic variation between parental lines affect both the power and accuracy of the QTL study (Mason et al., 2013). In both populations, when parents showed significantly different values in different traits, the RIL population exhibited continuous variance and transgressive segregation for all characteristics. The fundamental goal of durum wheat breeding projects worldwide has been to develop durum wheat cultivars with high grain yield and high-quality traits than can perform well across different environments. Based on our results, the average yield in POP2 was higher than in POP1. One of the reasons could be using two cultivar lines as parents in POP2, while in POP1, one of the parents was an experimental line with high quality and low potential yield. The higher range for yield in the Langdon trials could be the result of having lower temperatures at Langdon than at the Williston location. Stone and Nicholas (1995), reported higher temperatures (between 28 to 32 °C) can decrease yield by 20%. The higher temperatures in Williston could reduce the range and total yield in both populations (Tables 2.1 and 2.2). In other studies, it was shown that high

temperatures could affect several quality traits, such as dough properties, grain moisture, kernel weight, and test weight (Edwards et al., 1989; Panozzo et al., 2001). In POP1, in both locations, Joppa had a higher yield than the experimental line D121118, which shows that Joppa is a potential parent for developing lines with a high yield potential. In POP2, both parents had almost the same yield in all locations. In both populations, several progenies had higher yield than the parents.

Test weight is used as a criterion in grain grading, especially for wheat. The TWT of grains can be used to measure their soundness. When it comes to durum wheat, the TWT and semolina yield have a positive relationship. Low TWT would decrease the US grade classification in durum wheat (Cabas-Lühmann and Manthey, 2020). Williston trials had slightly higher TWT than Langdon trials possibly due to the effect of the environment. Delayed harvest could possibly decrease TWT (Gan et al., 2000). Cabas-Lühmann (2017) reported delaying harvest time in ND could reduce TWT. The possible reason for having lower TWT during delayed harvest is the amount of dew in the morning (McCaig et al., 2006), which is common in Langdon during harvest time. The lower TWT could decrease semolina yield and increase the amount of ash in the semolina. Durum lines with high ash scores have dimmer pasta products (Wang and Fu, 2020). Just like the yield data, the TWT for Joppa was higher than D121118 in POP1, and a few entry lines were out of the range of both parents. In POP2, Carpio had higher TWT than Mountrail and potentially produced lines with higher TWT in this population.

For YPC, in POP1, Joppa had a higher YPC than D121118. The population from the cross between the two had a continues distribution with transgressive segregates. Some progenies having YPC higher than the high parent and some lower than the low parent, which indicates YPC is a complex trait with a quantitative nature. Similar results were reported by Colasuonno et al. (2019).

Similar to POP1, in POP2, Carpio had higher YPC than Mountrail and the population had continuous distribution with transgressive segregates for YPC. Yellow pigment content in POP1 in Langdon was higher than Williston. However, in POP2, the Williston location had higher YPC than Langdon. Previous studies reported environment could affect YPC. Clarke et al. (2006) reported that high temperatures could decrease the amount of YPC. However, Rharrabti et al. (2003), reported that high temperature did not affect YPC. Reports from other studies showed that YPC is controlled more by genotype than environment (Borrelli et al., 2003; Digesu et al., 2009; Taghouti et al., 2010).

Soluble brown pigment reduces the quality of pasta. In this study, SBP frequency distribution shows that most progenies have a relatively low amount of SBP. In general, both populations had low SBP and it was influenced by the environment. Williston trials showed higher amount of SBP than Langdon trials (Tables 1.1 and 1.2); however, these results do not support Cabas-Lühmann and Manthey (2020) results that indicated high humidity could increase the amount of SBP.

To our knowledge, no transgressive segregates have been reported in the literature for SBP. However, in our study several lines had higher SBP than the high parent and some were lower than the low parents. Knox et al. (2012) reported the complexity of traits such as preharvest sprouting in durum wheat could lead to transgressive segregation in progenies. The genetic nature of SBP is not as clear as it is for YPC; more research is needed to find the inheritance and genetic control of SBP.

One of the main reasons for having brown color in pasta is enzymatic activity such as PPO activity (Kobrehel et al., 1974). Polyphenol oxidase that remains after milling in semolina could make a brown color in pasta products (Demeke et al., 2001; Rani et al., 2001; Verlotta et al., 2010).

In POP1, both parents Joppa and D111218 had the same PPO levels which may have resulted in a small variation in this population. Several lines in this populations had very low PPO compared to the parents. Epistatic effects may have caused this observation.

Environmental effect was different for both populations. In POP2, both locations have almost the same amount of PPO, which indicates environments had less effect on PPO than genotype in this populations. However, in POP1, environment had a major effect on PPO. Several studies reported similar results as POP1, which PPO is more related to environment than genotype (Park et al., 1997; Feillet et al., 2000). More research is needed to find the relationship between environment and PPO activity in durum wheat.

Peroxidase enzyme activity is a complex trait, and few studies have been conducted to find the nature of this trait. Peroxidase activity could change the color and flavor of products (Burnette, 1977) and is one of the main enzymatic reasons for making a brown color in pasta products (Kobrehel et al., 1974).

Feillet et al. (2000) reported genotype has more effect on POD than the environment. Although based on our results, POD was higher in Williston than Langdon (Tables 1.1 and 1.2). In both populations, having progenies with significantly higher or lower amounts of POD than the parents indicate

the complex nature of this trait. Knox et al (2012) explained that complex traits such as yield or early maturity in durum wheat give rise to transgressive segregation and have progeny with extremely high or low phenotypes. The other reason for having an extremely high or low amounts of POD could be due to epistatic effects and will be discussed in chapter three.

Conclusion

In this study we developed two RIL populations by crossing cultivar Joppa with experimental line D121118 (population one) and cultivars Mountrail and Carpio (population two). In POP1, Joppa has a high YPC and low PPO activity, while experimental line D12118 has a low YPC and low PPO activity. In POP2, Mountrail has low YPC and PPO activity, while Carpio has high YPC and PPO activity. Yield, TWT, and all of the color-related traits revealed significantly different values between the parental lines. The RIL populations demonstrated continuous variation and transgressive segregation for all the traits, implying polygenetic inheritance and contribution of favorable alleles for all traits by all parental lines. In both populations, several lines had higher yield, TWT, and YPC than the check cultivars (ND-Riveland). Also, several lines were lower in SBP, PPO, and POD enzyme activity in both populations than all parents and checks. Two inbred lines in POP1 (Entry lines 32 and 78) with high YPC and low SBP and three entry lines (entry lines 1, 82, and 101) in POP 2 with high YPC were identified. The RILs with combinations of desirable alleles from both parents at specified loci could be useful in breeding programs to improve pasta color. Our results indicate Carpio and Joppa and a few selected experimental lines could be excellent sources to enhance yellow color in durum wheat breeding programs.

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CHAPTER 3. IDENTIFICATION OF MULTIPLE STABLE QTL IN DURUM WHEAT USING A HIGH-DENSITY SNP-BASED LINKAGE MAP

Abstract

The breeding of durum wheat is being done for several reasons, one of which is to increase the yellow color of the pasta. In this study, two populations of 192 recombination inbred lines (RILs) were developed from two different crosses (POP1: Joppa × D121118 and POP2: Mountrail × Carpio) to identify quantitative trait loci (QTL) for four color-related traits, including total yellow pigment content (YPC), soluble brown pigment (SBP), polyphenol oxidase (PPO), and peroxidase (POD). The phenotyping of these traits was carried out at two locations in North Dakota, USA. The genotyping for RIL populations was conducted using the wheat Illumnia iSelect 90K SNP assay. Two high density genetic maps consisting of 2,391 (POP1) and 3,471 (POP2) SNP markers were constructed, with an average marker density of 0.93 (POP1) and 0.49 (POP2) cM/marker. QTL mapping identified 19 additive QTL (A-QTL) in POP1 (12 A-QTL for YPC, three for SBP, and four for PPO) and 12 A-QTL in POP2 (four for YPC, two for SBP, three for PPO, and three for POD). A total of 370 minor digenic epistatic (DE-QTL) associated with all four traits in both populations were found. These A-QTL were distributed across the whole durum wheat genome except chromosomes 3B, 4B, and 6A. The major A-QTL were detected on chromosomes 5A (*D1.SBP5A.ndsu*) for SBP, 5B (*D1.YPC5B.ndsu*) and 6B (D1.YPC6B.ndsu) in POP1 for YPC. In POP2, major A-QTL were on chromosomes 2A (*D2.PPO2A.ndsu*), 3A (*D2.POD3A.ndsu*), and 7A (*D2.YPC7A.ndsu*) for PPO, POD, and YPC, respectively. The phenotypic variation explained (PVE) by these QTL varied from 15 to 54%. The information provided in the current study could be employed in marker-assisted selection (MAS) to increase selection efficiency and to improve the color in durum wheat.

Introduction

Durum wheat (*Triticum turgidum* L. *ssp*. *durum* Desf.) is one of the main crops for human consumption, and it is essential for the manufacturing of pasta, couscous, freekeh, and bulgur (laido et al., 2013; Hu et al., 2015; Maccaferri et al., 2019; NASS, 2021). Semolina from durum wheat is also one of the key ingredients in making flatbread in Mediterranean countries (Fadda et al., 2010; Gallo et al., 2010; Farbo et al., 2020). The color of pasta can range from bright yellow to a dingy brown. Pasta's brightness and yellowness are important quality indicators for both producers and customers (Troccoli et

al., 2000). It is widely believed that the bright yellow color of semolina and pasta is an indication of superior quality and nutritional content. Releasing a durum wheat that produces pasta products with high yellow color can be an important goal for breeders.

About 12,000 years ago, tetraploid wheats were domesticated throughout the Mediterranean area, including southern Europe, North Africa, and Southwest Asia (Bozzini, 1988). In the Fertile Crescent, this domestication process changed wild emmer (*T. turgidum* L. subsp. *dicoccoides*) into cultivated emmer (*T. turgidum* L. subsp. *dicoccom*) (Zohary et al., 2012; Ren et al., 2013; Gioia et al., 2015). The genome of durum wheat is AABB, which was generated from a spontaneous hybridization between *T. urartu* (AA genome, 2n = 14) and an ancient relative of Aegilops speltoides (donor of BB genome) (Peng et al., 2011). Durum wheat is the only tetraploid wheat cultivated on a large scale (Rapp et al., 2019).

Pasta prepared from high-quality durum wheat varieties has a bright yellow color and is resistant to surface disintegration and stickiness after cooking (Aalami et al., 2007). The bright yellow color of pasta is a characteristic that consumers highly regard. The yellowness in semolina is determined by the proportion of yellow to brown components in the flour mixture (Borrelli et al., 2008).

Yellow pigment content is one of the most important components to affect the end-used pasta color (Borreli et al., 2003). In the past few decades, YPC in cultivars has been improved. Recently released durum cultivars with high yellow pigment color shows the importance of this trait in durum breeding programs (Digesù et al., 2009). According to several studies published on the nature of carotenoids, lutein and its stereoisomer zeaxanthin, and two non-pro-Vitamin A xanthophylls are the predominant carotenoids in durum wheat (Adom et al., 2003; Panfili et al., 2004). It is reasonable to predict that the presence of carotenoids will result in a more appealing color for customers (Leenhardt et al., 2006); however, enzyme activities such as lipoxygenase (LOX), PPO, and POD can change the final color of pasta products (Borrelli et al., 2008).

Borrelli et al. (1999) reported the total loss of carotenoids after milling is around 8%. Enzymes such as LOX need oxygen to decrease the carotenoids. The first step of pasta processing, kneading, can bring oxygen with water into the sample and lead to the loss of color (Delcros et al., 1998; Borrelli et al., 2003). Because oxygen affects lipid oxidation in the dough and reduces the yellow color in end-use

products, most industries create a vacuum during pasta extrusion to reduce the presence of oxygen. (Hidalgo et al., 2010). As a family of enzymes, LOX is responsible for breaking down lipids, resulting in compounds that may be detrimental to pasta color and flavor (Hessler et al., 2002). The activities of the enzymes POD and PPO in durum wheat are responsible for the brown color of pasta (Kobrehel et al., 1974; Taha and Sagi, 1987). Only PPO enzymes are active during pasta preparation (Feillet et al., 2000), while POD does not respond due to a lack of their principal substrate, hydrogen peroxide (Delcros et al., 1998; Icard-Vernie're et al., 1999). However, because POD may oxidize carotenoid pigments, it could play an interesting role in color expression (Iori et al., 1995; Borrelli et al., 2003, 2008). Peroxidase activity was discovered to be positively connected with the brown index of pasta products (Kobrehel et al., 1972). Leenhardt et al. (2006) conducted research and found that einkorn has three to five times lower POD activity than durum and spring wheat. In a study conducted by Borrelli et al. (2008), POD enzyme activity from outer layers of endosperm to the center of the kernel decreased considerably. They found the biggest decrease of POD activity was often reported in cultivars with already low starting levels of this enzyme. This result supported a study by Rani et al. (2001), which found POD activity was about five times lower in semolina than whole wheat flour. They found that POD activity was often abundant in the outer layers of the endosperm, which was removed during milling. The enzyme PPO has been linked to discoloration in raw Asian noodles and other wheat products (Kruger, 1994; Morris, 1995). Consumers dislike darkened durum wheat products; thus, there has been a concerted attempt to reduce levels of PPO activity in durum wheat germplasm (Fuerst et al., 2006). Like POD, bran in durum wheat kernels has the highest PPO percentage in grain; thus, whole wheat flour has more PPO than semolina (Hatcher and Kruger, 1993; Hatcher et al., 1997). Several studies (Irvine and Winkler, 1950; Kobrehel et al., 1974; Taha and Sagi, 1987; Borrelli et al., 1999; Rani et al., 2001; Aalami et al., 2007) researched the relationship between PPO and color in pasta. High level of YPC alone does not provide a pasta with a desirable yellow color; factors such as enzyme activities (PPO and POD) can affect the color as well.

The basic process of PPO-meditated discoloration is as follows (Fuerst et al., 2006): The hydroxylation of monophenols to o-diphenols ("monophenolase" activity) and the oxidation of o-diphenols to o-quinones ("diphenolase" activity) are catalyzed by PPO (Steffens et al., 1994). Then, the resulted products react with various functional groups, including amines, thiols, and phenolics, to produce a variety

of complex colored compounds such as melanin (Whitaker and Lee, 1995). While, several researchers have indicated that discoloration still happens in durum wheat with zero PPO activity (Mares and Panozzo, 1999); Fuerst et al. (2006) reported that in durum wheat with high PPO activity, the brownness of noodles was three to five times more than durum wheat with low enzyme activities.

The time-consuming nature of phenotyping methods for the high yellow color in pasta may lengthen time to release new cultivars. This is because color testing could be expensive, and a considerable volume of grain is required for the testing of semolina and pasta products. In the case of durum wheat, performing these tests at a late point in the breeding program frequently leads to superior wheat lines in most aspects but cannot be released due to poor yellow color. Due to these issues, many breeders rely on genotype-based selection to discover color-related QTL and associated markers, with the goal of using such markers for marker-assisted pasta color improvement (Mares and Campbell, 2001; Pozniak et al., 2007; Howitt et al., 2009; Zhang et al., 2009; Blanco et al., 2011; Roncallo et al., 2012; Kumar et al., 2013).

Bi-Parental mapping (also known as QTL mapping) and other marker assisted selection (MAS) techniques are being developed using molecular breeding techniques to make it easier and faster to release superior cultivars with a high Yellow Index (YI). QTL-mapping is based on the idea that genes and markers segregate through chromosome recombination. When compared to genes and markers that are far apart, genes and markers that are close will co-segregate and transmit from parent to progeny more frequently (Paterson, 1996). The choice of two parental lines significantly impacts the power and accuracy of QTL detection (Jansen et al., 2001). Allelic polymorphism and phenotypic diversity between parental lines are important factors in determining both power and accuracy (Mason et al., 2013).

Several types of research were conducted to find the position of color-related traits in the durum wheat genome (Rharrabti et al., 2003; Pozniak et al., 2007; Taghouti et al., 2010). Quantitative inheritance mixed with additive effect genes explains the inheritance of the carotenoid content. The primary QTL for carotenoid content is located on chromosome 7, whereas other minor QTL can be discovered in all durum wheat genomes (Colasuonno et al., 2019). The essential gene in the carotenoid system is the *PSY* gene, which codes for the enzyme phytoene synthase (Gallagher et al., 2004; Li et al., 2008). He et al. (2008) investigated three unique environments and discovered that two *Psy-A1*

haplotypes on chromosome 7A explained 20-28% of the phenotypic variability in YPC. Giraldo et al. (2016) analyzed durum wheat landraces and cultivars and found two significant markers associated with YI on chromosome 3B, which explained 14-17% of PVE. Also, they found minor QTL on chromosome 3B have more impact on YPC than *PSY* gene on chromosome 7B. Many minor QTL have been detected on all of the chromosomes of durum wheat, indicating that the inheritance of YPC is relatively complex (Ficco et al., 2014).

Several experiments to test the activity of the POD enzyme have been conducted, with various results reported (Asins and Perez de la Vega, 1985; Bosch et al., 1986; Liu et al., 1990; Ficco et al., 2014). These studies show that minor QTL in the wheat genome determine kernel POD activity. A variety of minor QTL are found on chromosomes 1, 2, and 3, each of which is more active in a different part of the durum wheat plant. *Per-1* is expressed in the coleoptile tissue. While *Per-2* is expressed in the root tissue. *Per-3* is active in embryonic tissue, whereas *Per-4* is expressed in the endosperm and could be found on chromosomes 7A, 7D, and 4A. Two significant QTL on chromosomes 3A and 4B explain 5.3-21.2% of PV for POD enzyme activity (Wei et al., 2015). Peroxide could be found in endosperm and embryo, with endosperm POD QTL located on chromosomes 4A and 7A, while QTL responsible for embryo POD are located on chromosomes 3A and 3B (Bosch et al., 1987).

The essential genes encoding the PPO enzyme in durum wheat may be identified on chromosome 2A. The *PPO-A1* gene, found on chromosome 2A, accounts for a considerable amount of phenotypic variation in wheat (Watanabe et al., 2006). The *PPO-B2* gene has also been linked to other PPO genes and has been demonstrated to influence PPO activity. To find QTL to determine the PPO activity in durum wheat, Watanabe et al. (2006) conducted research and found chromosome 2A is the significant location associated with PPO enzyme activity. Their result was confirmed by He et al. (2007), which found chromosome 2A has a significant role in PPO activity. In another study by Taranto et al. (2015), two important markers (MG08 and MG33) for PPO in chromosome 2B were identified.

There appears to be a scarcity of research available about the genetic control of SBP. Although several studies were conducted to find the QTL and markers associated with the color-related traits, none of them reported the relationship between them. Most of the research relied on a small number of molecular markers, due to the low density of genetic linkage maps used for color-related traits in the past.

The wheat Illumina 90K iSelect assay (Wang et al., 2014) was utilized in the current work to detect gene-phenotype relationships for YPC, SBP, PPO, and POD enzyme activity.

Despite the fact that many QTL have been discovered in crops, the vast majority have not been employed in breeding efforts (Bernardo, 2008). Because advantageous QTL allele may already be fixed in breeding programs, significant marker-trait associations may not have predictive value in breeding germplasm. As a result, employing breeding lines to detect QTL would considerably improve the utility of QTL identification for genotype-based selection. A decrease in enzymatic activity and SBP with an increase in YPC could result in higher yellow color for pasta products. Durum breeding programs can release cultivars with a higher YPC, lower SBP and enzyme activity levels, and guarantee a high yellow desirable color in end products.

In the current study we used four different parents from the North Dakota State University (NDSU) durum breeding program to find the markers associated with YPC, SBP, PPO, and POD. This chapter discusses the genotyping of two different population linkage maps. The objectives of this chapter were: 1) Identify the molecular markers associated with four different color-related traits (YPC, SBP, PPO, and POD enzyme activities). 2) Study the genetic relationship between enzyme activities and yellow pigment content in pasta.

Material and Methods

Plant Material

As described in chapter two, two RIL mapping populations were developed from two crosses in the durum wheat breeding program at NDSU. The first cross (POP1) was developed from crossing the high YPC and low PPO activity durum cultivar “Joppa” (Elias and Manthey, 2016) with the low YPC and low PPO activity experimental line D121118. The second RIL population (POP2) was developed by crossing the durum cultivar "Mountrail" (Elias and Miller, 2000), which has low YPC and low PPO activity, with the durum cultivar "Carpio" (Elias et al., 2015), which has high YPC and high PPO activity.

Joppa was chosen because of its high grain yield potential, disease resistance, and excellent end-use attributes. Joppa was developed from crossing the durum cultivar "Maier" with the durum experiment line D97643. D121118 is a high-quality experimental line developed in the durum wheat-breeding program at NDSU.

Carpio was released because of its high yield potential, good quality, and disease resistance. Carpio was developed from a cross between two durum experimental lines (D95580 and D95595). Mountrail has excellent yield potential and good quality. Mountrail was developed by crossing the durum experimental line D8479 with the durum cultivar Renville (Elias and Miller, 2000).

The single seed descent (SSD) method was used to develop both RIL populations in this study. The original crosses were made in the winter of 2016. In spring greenhouse of 2017, F_1 seeds were planted; F_2 seeds were harvested and advanced as SSD to generate the subsequent generations. To shorten the duration and increase the number of seeds, one spike per plant was picked and planted as $F_{4:5}$ head rows in the 2019 winter nursery in New Zealand. $F_{4:6}$ lines were harvested in bulk and planted in two replicates at two locations (Langdon and Williston, North Dakota [ND]).

In each population, a total of 196 RIL (including parents and checks) were used in phenotypic evaluation of color-related traits. We used two-row plots in Langdon and five-row plots in Williston to evaluate all traits in both populations. The experiment design was a simple lattice for both populations. We used the durum cultivars "Divide" (Elias and Manthey, 2007) and "ND-Riveland" (Elias and Manthey, 2019) as checks. All parents were also included in the tests.

Phenotyping

Yellow Pigment Content (YPC)

The YPC was determined using a modified AACCI approved-method 14-50.01. In the modified method, the sample size was 4 g of whole wheat flour instead of 8 g. Water-saturated n-butanol reagent (WSB) (20 ml) was combined with 4 g of whole wheat flour (5:1 ratio). After shaking for two minutes on a vortex mixer and resting for one hour, the samples were centrifuged for five minutes at 18,514 x *g* relative centrifuge force (RCF) (Eppendorf 5810R centrifuge, Rotor: F-34-6-38, Radius: 11.5cm). The absorbance of the supernatant at 436 nm was measured using a spectrophotometer (VWR UV-1600 PC Spectrophotometer). Measurements per extracted sample were converted to yellow pigment concentration (µg/g or ppm) using the β-carotene extinction coefficient 1.6632.

Soluble Brown Pigment Content

The SBP was determined using a modified method reported by Matsuo and Irvine (1967). Whole wheat flour (2 g) was combined with 4 ml of deionized distilled water. The mixture was mixed twice on a

vortex mixer, then rested for five minutes before centrifuging for six minutes at 18,514 x *g* relative centrifuge force (Eppendorf 5810R centrifuge, Rotor: F-34-6-38, Radius: 11.5cm) (RCF). Then, the supernatant (1.5 ml) was centrifuged for 10 min in an Eppendorf tube. The absorbance of the supernatant at 400 nm was measured using a spectrophotometer (VWR UV-1600 PC Spectrophotometer). As a final measurement, the OD_{400} was recorded.

Polyphenol Oxidase Enzyme Activity

The activity of PPO was evaluated in this study using an AACCI-approved method 25-85.01. Five mm of L-3, 4 dihydroxyphenylalanine (L-DOPA) in 50 mm of 3-(N-morpholino) propanesulfonic acid (MOPS) buffer pH 6.5, 0.02% solution Tween-20 (1.5 ml solution) was added to the whole wheat flour (200 mg) in a micro-centrifuge tube. The tubes were placed on an orbital shaker (Glas-Col, Terre Haute, IN, USA) and rotated for one hour at room temperature to appropriately initiate the reaction. The samples were then centrifuged for two minutes at 14,000 x *g*. A VWR UV-1600 PC Spectrophotometer was used to measure the absorbance of the supernatant at 475 nm. A control sample was included in each sample, which was treated with a solution that did not contain L-DOPA. The absorbance of each sample was subtracted from the absorbance of the control. The enzyme activity was measured in ΔA475/min g of material with one unit of enzyme activity equaling a change in absorbance unit per minute.

Peroxidase Enzyme Activity

Peroxidase activity was measured using the technique described by Honold & Stahmann (1968) and Fraignier et al. (2000), with slight modifications. A total of 150 mg of whole wheat flour was combined with 1.5 ml of 50 mM phosphate citrate buffer pH 4.6 (1:10 w/v) and shaken for two hours at room temperature in an orbital shaker (Glas-Col, Terre Haute, IN, USA). After finishing the rotation, the samples were centrifuged for 10 minutes at 14,000 x *g*. Afterwards, 1.5 ml of a solution containing five mM guaiacol, ten mM H_2O_2, and 50 mM phosphate citrate buffer (5 pH) was added to the supernatant. To quantify the POD enzyme activity, the oxidation of guaiacol to tetrahydroguaiacol in the presence of H_2O_2 was measured spectrophotometrically (VWR UV-1600 PC Spectrophotometer) at 470 nm for a minute, recording every 15 seconds. Units per gram of dry mass (U/g) was used to calculate total activity. One unit of enzyme activity was defined as a change in absorbance unit per minute.

Statistical Analysis

The two RIL populations were planted in both locations (Langdon and Williston, ND) using a simple lattice design. Each location was considered as a separate environment (two environments total), each with the two populations. Environments were treated as random effects, whereas genotypes were treated as fixed effects. When the ratio (Fmax) of the effective error variances for each trait was less than 10-fold, the environment was declared homogenous (Tabachnik and Fidell, 2001). When error variances were homogeneous, a combined analysis across locations was conducted. Statistical Analysis System (SAS) version 9.4 for Windows (SAS Institute Inc., Cary, NC, USA) was used to analyze the data. LSMeans were computed, and mean separation was conducted with a macro statement at a 95% level of confidence (F tests: $P<0.05$).

Genotyping

The greenhouse at NDSU was used to plant the seeds of each genotype. Each individual`s DNA was extracted from two inches of leaf tissue. After harvesting each line, the collection equipment was cleaned with ethanol to prevent DNA contamination. The leaf tissue was collected in a 96 well block. Each of the 96-well blocks included 2.5 mg of silica gel. In order to conduct molecular testing, the samples were sent to the United States Department of Agriculture (USDA)-ARS Cereal Crop Genotyping Laboratory in Fargo, ND. The protocol for extracting DNA from wheat can be viewed at: http://wheat.pw.usda.gov/GenotypingLabs/fargo.html.

The DNA samples were genotyped at the same laboratory where the DNA extraction was performed, using wheat 90k SNP chips (Wang et al., 2014). SNP markers were then called in the same manner described by Wang et al. (2014), using the Genome Studio Polyploid Clustering Module v1.0 software (www.illumina.com). In total for both populations, 5,862 polymorphic SNP (Tables 3.1 and 3.2) markers between parents were discovered from a total of 81,582 SNP markers from the wheat Illumina 90K iSelect assay (Wang et al., 2014). Markers with a high number of missing data (15%), low minor allele frequency (<0.3), conflicting results in three replicates of each parental genotypes, and substantial segregation distortion ($\chi 2$ goodness-of-fit statistic, $p< 0.001$) were removed. In diploid organisms there are three clusters of bi-allelic loci. The three genotypic classes AA, AB, and the BB clusters represent each of them. Two clusters (AA and BB) are expected for each segregating marker assay in an F_6 RIL

population. In order to ensure that all possible errors related to cluster assessment were corrected, each genotype was individually checked and adjusted as needed. Afterward, the final genotyping data were exported to an Excel file, then used to generate the genetic map.

A combination of Joinmap 5 software (Van ooijen, 2006), IcIMapping software 4.2 (Meng et al., 2015), and MSTmap software (Wu et al., 2008) was used to do the linkage analysis for the 5,862 SNP markers in this study (2,391 markers in POP1 and 3,471 markers in POP2). The initial step was to choose 140 anchor SNP markers for all 14 durum wheat chromosomes using two high-density SNP consensus maps (Wang et al., 2014; Maccaferri et al., 2015) as the reference maps. For choosing anchor markers, five to fifteen SNP markers across the whole length of each chromosome were chosen. In both populations, all markers including the 140 anchor markers, with a minimum Logarithm of the Odds (LOD) score of 5.0 (starting at 20.0) and a maximum distance of 40 centimorgans (cM), were placed into the 28 linkage groups using Joinmap. To estimate the distance of all 14 durum wheat chromosomes and avoid reversed maps, MSTmap software was used with a cut-off at $p < 0.000001$, the maximum distance of 15 cM between markers, grouping LOD criteria of 5.0, and a minimum linkage group size of 2 cM. Kosambi's genetic mapping tool was used to calculate genetic distances between markers (Kosambi, 1944). The genetic linkage groups were compared with the Wang et al. (2014) high-density SNP consensus map to validate the accuracy of the markers order. MapChart software version 2.2 was used to create the final genetic linkage maps and related graphics (Voorrips, 2002).

Quantitative Trait Loci Mapping

The Inclusive Composite Interval Mapping (ICIM) approach, which is included in the software QTL IciMapping V 4.2 (Meng et al., 2015), was used to perform additive QTL (A-QTL) analysis on individual environmental data as well as mean data across environments for each population. The ICIM statistical method starts with a stepwise regression, then a one-dimensional scanning for mapping additive effects, and finally a two-dimensional scanning for mapping epistasis effects. Stepwise regression ($p < 0.001$) with simultaneous consideration of all marker information was used in QTL IciMapping. For every A-QTL, the step size was retained at the default of 1.0 cM. One-LOD drop-off from the estimated A-QTL was used to establish left and right confidence intervals (Meng et al., 2015). The LOD threshold values for detecting significant A-QTL were determined using a permutation test with

1,000 iterations at Type I error of 0.05 and all A-QTL found over the LOD threshold value were reported. For each linkage group, the markers in unique loci position were employed in IciMapping. The cumulative distance was used to define linkage group markers.

The digenic epistatic QTL (ICIM-EPI) approach was used to find additive-by-additive epistatic interactions or digenic epistatic QTL (DE-QTL) for each of the color associated traits inside each environment, as well as across all environments (Meng et al., 2015).

A stable DE-QTL was defined as one that was identified across all environments. The DE-QTL was designed with a 5.0 cM step size. For DE-QTL, the probability utilized in stepwise regression was 0.0001. The LOD threshold values were kept at the default value of 2.5 to detect DE-QTL. The minimal LOD value for asserting significant A-QTL or DE-QTL was determined using a permutation test.

The QTL named to represent “D” as durum, “YPC, SBP, PPO, and POD” represent the traits, followed by the chromosome number, and “ndsu” as the institute name (e. g. *D1.YPC5A.ndsu*)

Result

In both populations, all of the color-related traits (YPC, SBP, PPO, and POD activity) showed significantly different values between the parental lines and entry lines. The RIL populations demonstrated continuous variation and transgressive segregation for all color-related traits, implying polygenetic inheritance and contribution of favorable alleles for color-related traits by all parental lines.

Genetic Linkage Map

For both populations, 5,862 SNP markers were selected for making the genetic linkage groups. Each population had 28 linkage groups, which covered all 14 durum wheat chromosomes. The linkage maps in POP1 covered a total genetic length of 2,232.13 cM, with an average marker distance of 0.93 cM (Table 3.1). Only one linkage group was found for chromosomes 1B, 2B, 4B, and 5A. Chromosomes 1A, 2A, 3A, 6A, 7A, and 7B had two linkage groups each. Finally, chromosomes 3B, 4A, 5B, and 6B were shown to have three linkage groups. There were 1,409 unique loci (61% of all markers) on the linkage map, with an average genetic distance of 1.55 cM between any two unique loci. The A-genome had significantly more markers (1,260) and higher genetic length (1,234.68 cM with an average distance of 0.98 cM between two loci) than the B-genome (1,131), which had a total of 997.45 cM and an average distance of 0.88 cM between two loci. Individual linkage groups ranged from nine (4B) to 282 markers

(6B-2). Furthermore, for the 4B and 6B-2 linkage groups, the number of unique loci in a linkage group ranged from 5 to 180.

For POP2, total map length was 1,714.16 cM, with an average distance between any two markers of 0.49 cM (Table 3.2). With an average distance of 0.99 cM between two unique loci, the 3,471 markers represented 1,711 unique loci (50%). The A-genome had a total of 1,423 markers with an average distance of 0.56 cM per locus, these loci markers cover a map length of 789.84 cM. The B-genome was mapped with a total of 2,048 markers with an average distance of 0.45 cM per locus, these loci encompass a map length of 924.32 cM. Only one linkage group was identified for chromosomes 4A, 4B, 5A, and 6B. There were two linkage groups on each of the chromosomes 1A, 2B, 3A, 3B, 6A, 7A, and 7B. Chromosomes 1B and 2A each had two linkage groups. Finally, there were four linkage groups on chromosome 5B. The number of markers in each linkage group ranged from 11 (2A-2 and 6A-2) to 447 (1B-1). Additionally, the number of unique loci in a linkage group ranged from 8 to 241 for the 2A and 1B-1 linkage groups, respectively (Table 3.4). The marker arrangement was reasonably consistent compared to the high-density SNP consensus map published by Wang et al. (2014) and Maccaferri et al. (2015).

Table 3.1. Distribution of the SNPs linkage map of durum wheat population derived from crossing Joppa to the experimental line D121118 (Population One).

Linkage group	No. of markers	No. of unique loci	Map distance (cM)	Map density (cM/marker)	Map density (cM/locus)
1A-1	130	76	86.53	0.67	1.14
1A-2	248	145	151.88	0.61	1.05
1B	122	59	142.90	1.17	2.42
2A-1	48	27	69.02	1.44	2.56
2A-2	77	48	27.69	0.36	0.58
2B	186	118	229.63	1.23	1.95
3A-1	118	68	168.11	1.42	2.47
3A-2	17	12	4.22	0.25	0.35
3B-1	122	79	109.34	0.90	1.38
3B-2	31	16	5.84	0.19	0.36
3B-3	15	5	10.24	0.68	2.05
4A-1	28	21	62.61	2.24	2.98
4A-2	104	73	85.69	0.82	1.17
4A-3	96	64	26.55	0.28	0.41
4B	9	5	28.58	3.18	5.72
5A	174	93	356.99	2.05	3.84
5B-1	37	16	53.83	1.45	3.36
5B-2	71	23	23.57	0.33	1.02
5B-3	95	34	70.02	0.74	2.06
6A-1	15	8	18.29	1.22	2.29
6A-2	139	90	115.77	0.83	1.29
6B-1	24	20	21.44	0.89	1.07
6B-2	278	180	166.52	0.60	0.93
6B-3	78	49	56.10	0.72	1.14
7A-1	36	21	54.12	1.50	2.58
7A-2	30	18	7.22	0.24	0.40
7B-1	41	20	67.90	1.66	3.39
7B-2	22	21	11.55	0.52	0.55
A genome	1260	764	1234.68	0.98	1.62
B genome	1131	645	997.45	0.88	1.55
Whole genome	2391	1409	2232.13	0.93	1.58

Quantitative Trait Loci Analysis

For the four traits assessed in this study (YPC, SBP, PPO, and POD enzymes), a total of 31 distinct A-QTL and 370 DE-QTL were detected in both populations. In both populations, the A-QTL were found to be dispersed across all durum wheat chromosomes (Figures 3.1 and 3.2), with the exception of chromosomes 3B, 4B, and 6A. The A-QTL were distributed across the durum wheat genome, with the A-genome having the greatest number of A-QTL (21), and the B-genome having the least amount of A-QTL (10) (Table 3.2). Of the 31 A-QTL, a total of seven A-QTL (or around 23% of the total) explained more than 10% of PVE and were classified as major A-QTL, whereas the remaining 24 A-QTL explained less than 10% of PVE and were labeled as minor A-QTL. There were a total number of 16 A-QTL discovered across locations were considered stable A-QTL.

In POP1, 267 DE-QTL were detected across the genome, and for POP2, a total of 103 DE-QTL were identified across the durum wheat genome. There are two major and stable A-QTL for YPC and one A-QTL for PPO that were common in both populations (Table 3.3).

Table 3.2. Distribution of additive-quantitative trait loci for population one (Joppa × D121118) and population two (Mountrail × Carpio) across durum wheat genome.

Population	Genome	A-QTL	YPC	SBP	PPO	POD
POP1	A genome	13	6	3	4	-
	B genome	6	6	-	-	-
POP2	A genome	8	1	1	3	3
	B genome	4	3	1	-	-

Table 3.3. Common additive-quantitative trait loci (A-QTL) in both populations.

Trait	Chromosome	A-QTL name
Yellow pigment content	5B	*D1.YPC5B.ndsu*
		D2.YPC5B.ndsu
	7A	*D1.YPC7AW.ndsu*
		D2.YPC7A.ndsu
Polyphenol oxidase	3A	*D1.PPO3AL.ndsu*
		D2.PPO3A.ndsu

Table 3.4. Distribution of the SNPs linkage map of durum wheat population derived from crossing Mountrail and Carpio (Population Two).

Linkage group	No. of markers	No. of unique loci	Map distance (cM)	Map density (cM/marker)	Map density (cM/locus)
1A-1	14	10	1.93	0.14	0.19
1A-2	113	60	66.15	0.59	1.10
1B-1	447	238	169.75	0.38	0.71
1B-2	287	119	59.88	0.21	0.50
1B-3	97	41	18.15	0.19	0.44
2A-1	119	68	69.50	0.58	1.02
2A-2	11	6	8.21	0.75	1.37
2A-3	26	19	14.39	0.55	0.76
2B-1	75	45	53.76	0.72	1.19
2B-2	61	38	29.57	0.48	0.78
3A-1	66	21	19.87	0.30	0.95
3A-2	197	97	97.65	0.50	1.01
3B-1	14	10	9.49	0.68	0.95
3B-2	182	61	112.37	0.62	1.84
4A	110	62	148.33	1.35	2.39
4B	110	64	112.63	1.02	1.76
5A	159	94	157.82	0.99	1.68
5B-1	85	57	86.42	1.02	1.52
5B-2	97	67	17.68	0.18	0.26
5B-3	107	37	41.85	0.39	1.13
5B-4	50	18	28.55	0.57	1.59
6A-1	317	140	59.52	0.19	0.43
6A-2	11	9	8.61	0.78	0.96
6B	124	52	19.03	0.15	0.37
7A-1	209	122	106.60	0.51	0.87
7A-2	71	41	31.24	0.44	0.76
7B-1	201	89	149.88	0.75	1.68
7B-2	111	26	15.33	0.14	0.59
Sum	3471	1711	1714.16	0.49	1.00
A genome	1423	749	789.84	0.56	1.05
B genome	2048	962	924.32	0.45	0.96
Whole genome	3471	1711	1714.16	0.49	1.00

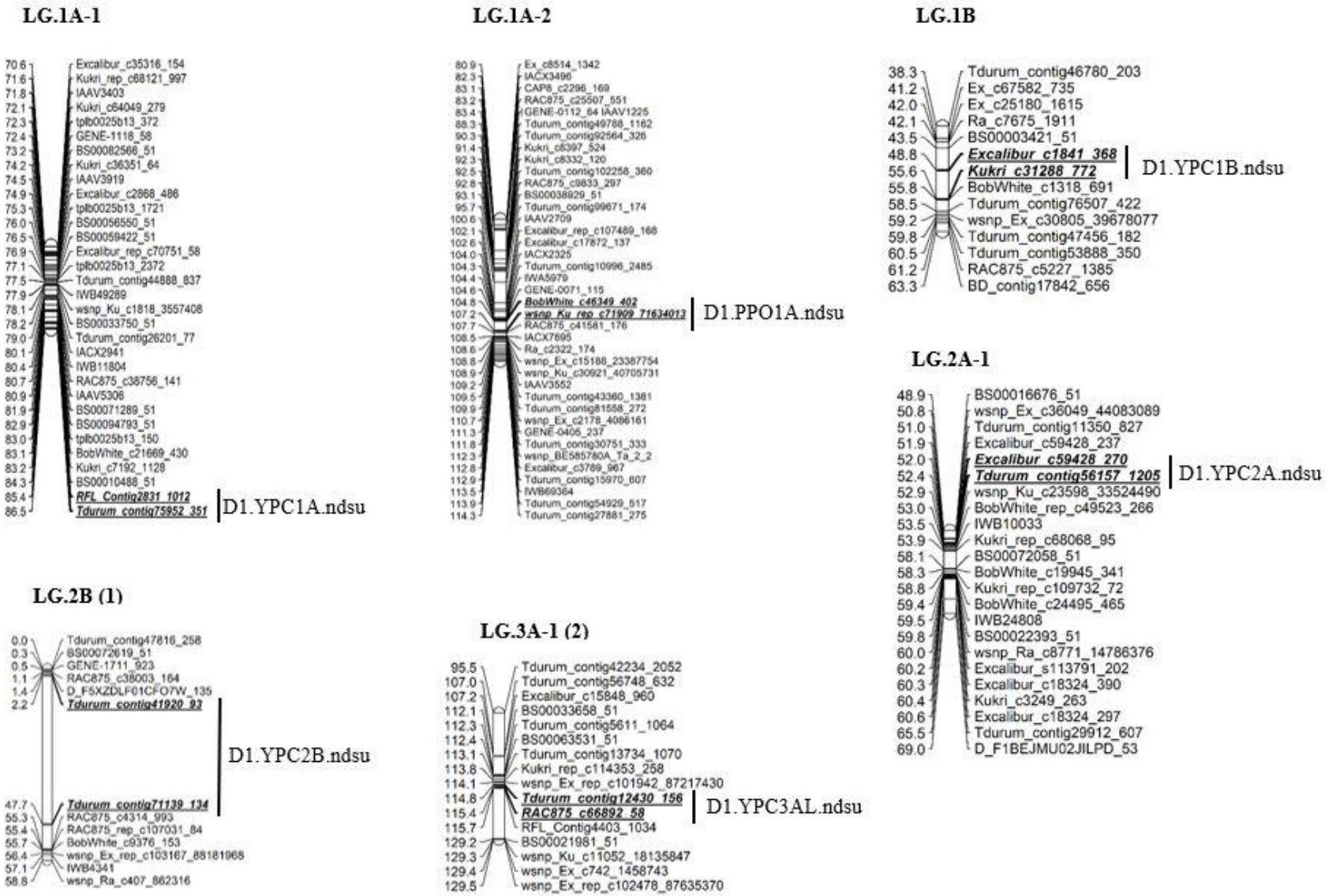

Figure 3.1. Additive-quantitative trait loci for color-related traits in durum wheat population derived from Joppa and D121118 (population one). QTL confidence intervals are indicated by bar bold and italic scripts.

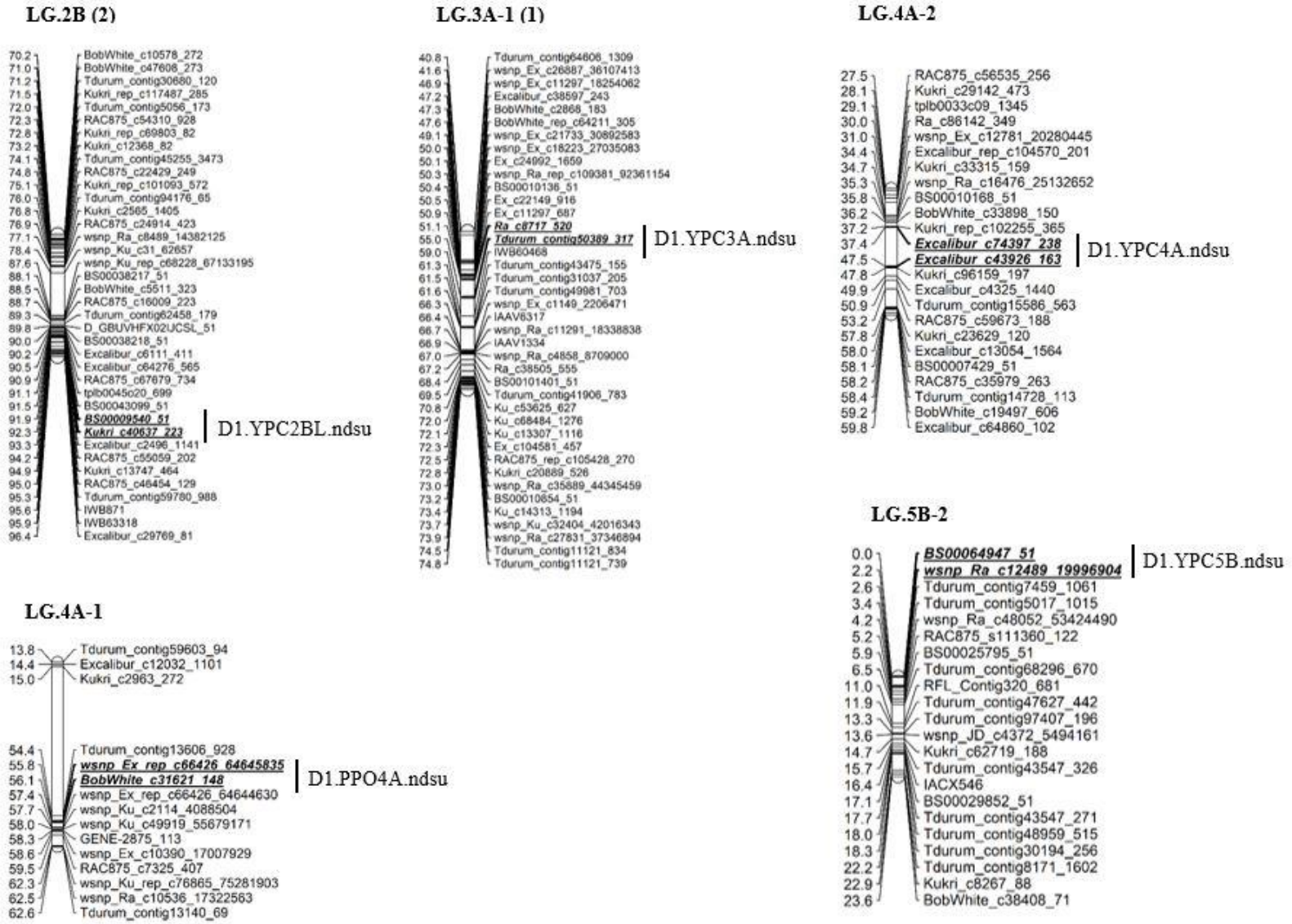

Figure 3.1. Additive-quantitative trait loci for color-related traits in durum wheat population derived from Joppa and D121118 (population one) (Continued). QTL confidence intervals are indicated by bar bold and italic scripts.

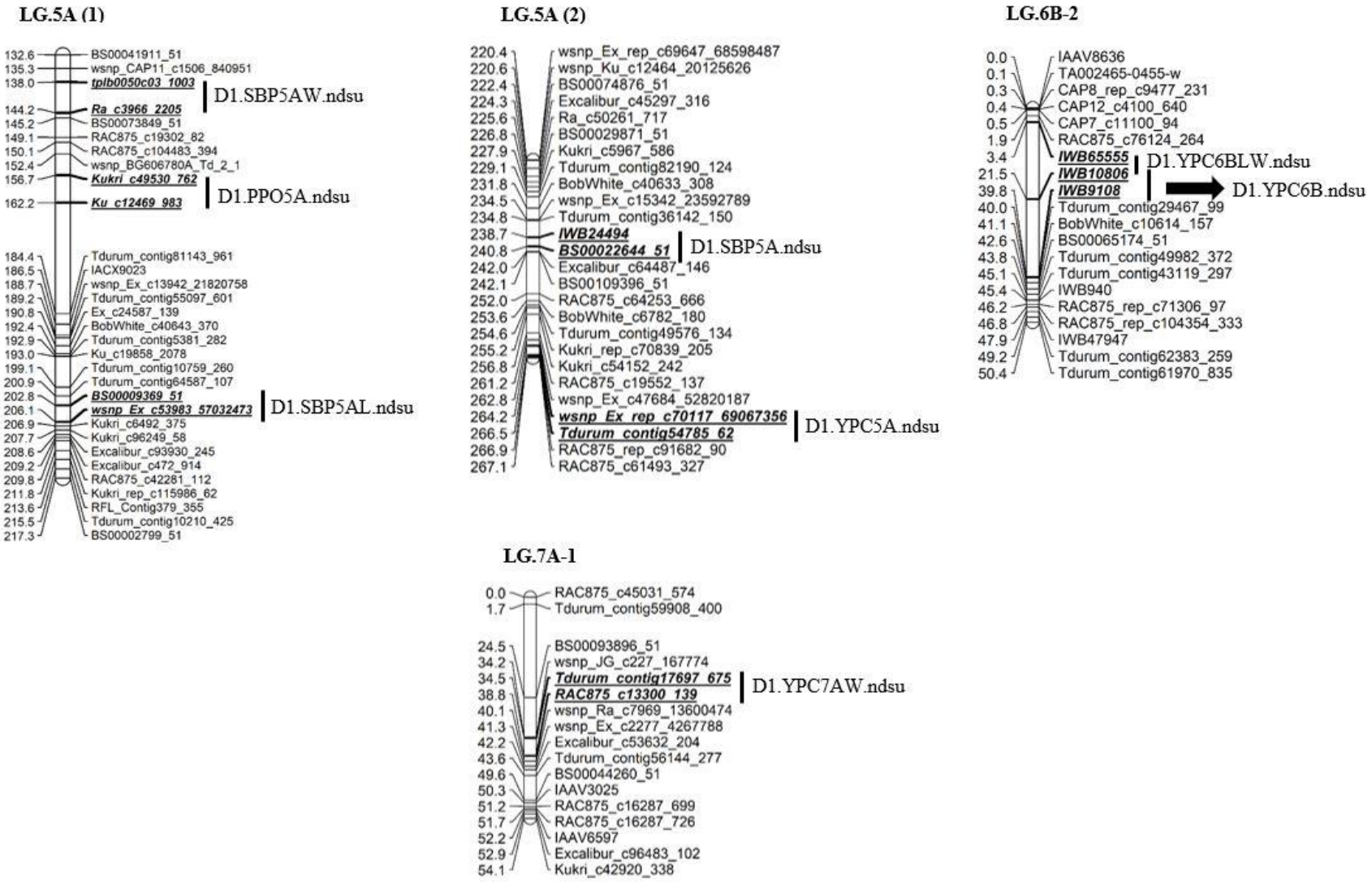

Figure 3.1. Additive-quantitative trait loci for color-related traits in durum wheat population derived from Joppa and D121118 (population one) (Continued). QTL confidence intervals are indicated by bar bold and italic scripts.

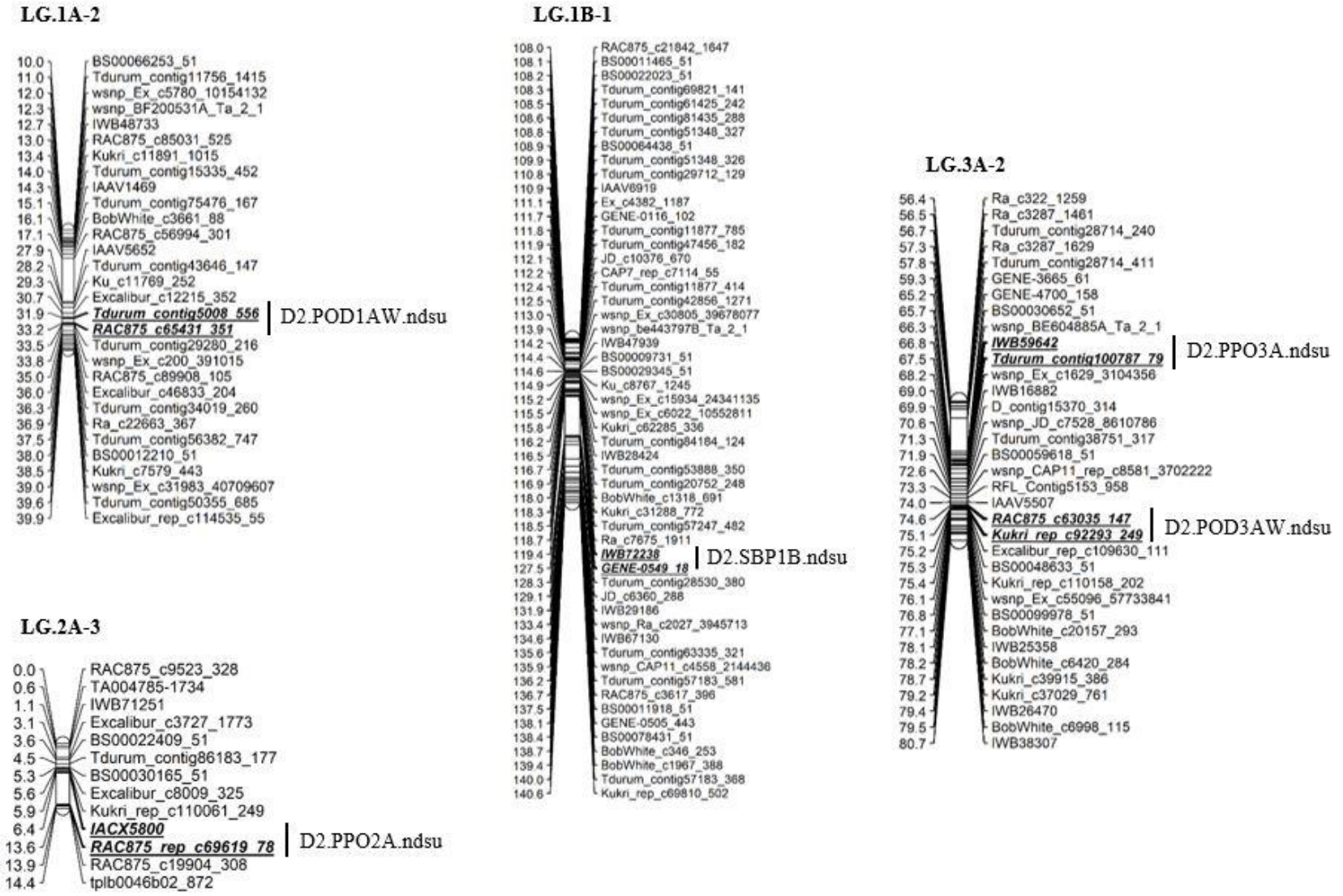

Figure 3.2. Additive-quantitative trait loci for color-related traits in durum wheat population derived from Mountrail and Carpio (population two). QTL confidence intervals are indicated by bar bold and italic scripts.

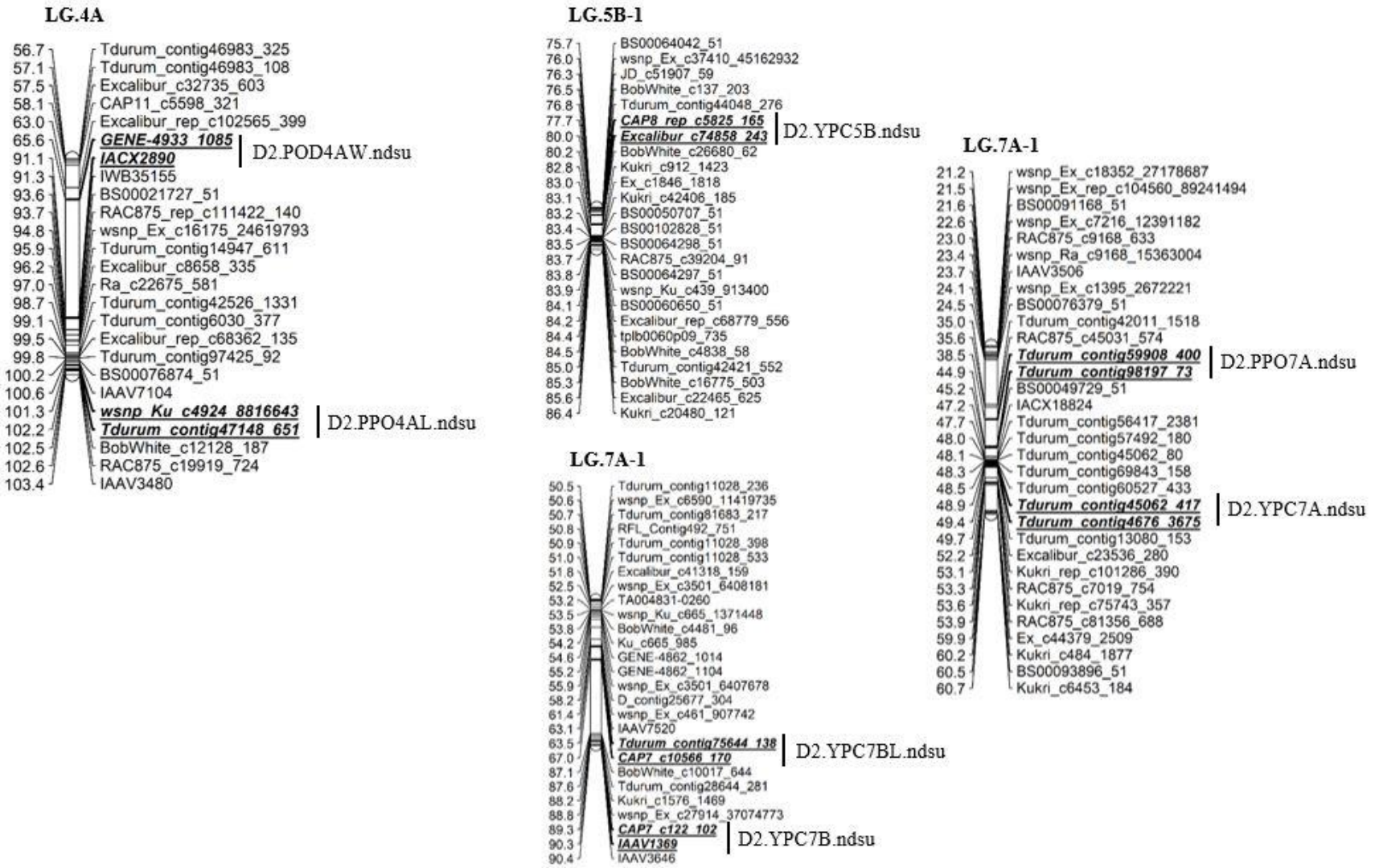

Figure 3.2. Additive-quantitative trait loci for color-related traits in durum wheat population derived from Mountrail and Carpio (population two) (Continued). QTL confidence intervals are indicated by bar bold and italic scripts.

Population One (Joppa x D121118)

Quantitative Trait Loci for Yellow Pigment Content

Two major stable A-QTL and five minor A-QTL linked with YPC were detected by QTL mapping across the two locations. For each separate location, in Langdon, three minor A-QTL were found to be linked with YPC, but in Williston, only two A-QTL were found to be associated with YPC (Table 3.5).

Quantitative trail loci *D1.YPC5B.ndsu* with a PVE of 16% had more effect on YPC than other A-QTL. The SNPs BS00064947_51 (left marker) and wsnp_Ra_c12489_19996904 (right marker) tightly flank this A-QTL (within 0.5 cM region [0.0 to 0.5 cM on 5B-2]), with a LOD score of about 16 (Table 3.5). The left flanking marker is co-segregated with two other SNPs (Tdurum_contig12776_278 and Excalibur_c13408_280), but the right-flanked marker is a unique locus. This A-QTL showed an additive effect of 0.224 ppm YPC in Langdon location and 0.225 ppm in Williston location, whereas this A-QTL in across locations had an additive effect of 0.214 ppm on YPC.

Another major and stable A-QTL in both locations for YPC was *D1.YPC6B.ndsu* on chromosome 6B-2 and position of 27 cM, with PVE of 11.6%, and LOD score of 7.2 (Table 3.5). This A-QTL had an additive effect of 0.17 ppm YPC across locations. The left SNP for this A-QTL was IWB10806 on 21 cM, and the right SNP was IWB9108 which was co-segregated with IWB10007 on 40 cM. However, this A-QTL in Langdon and Williston was found in the same chromosome but in a different position. The position was 17 cM in across locations, and SNPs IWB65555 (left marker) and IWB10806 (right marker) flanked this A-QTL within almost 17 cM. The left flanking marker was co-segregated with four SNPs (tplb0024i08_1067, BS00098103_51, BS00084314_51, and IWB7298), while the right flanking marker is a unique locus.

The main A-QTL was not the only one identified; five other A-QTL with small effects were found on each of the following chromosomes: 1A, 1B, 2A, 2B, and 4A (Table 3.5). The A-QTL on chromosome 1A-1 (*D1-YPC1A.ndsu*) was on 86 cM and explained 2.7% of PVE YPC and LOD of 2.5, and was identified only across locations. This A-QTL was tightly flanked with two SNPs within a 1.2 cM region (RFL_Contig2831_1012 [85.41 cM] and Tdurum_contig75952_351 [86.53 cM]). This A-QTL added 0.08 ppm to the YPC. The next minor A-QTL was found on chromosome 1B (Table 3.5). The A-QTL *D1.YPC1B.ndsu* was found across locations and explained 7.6% PVE with LOD of 6.9. This A-QTL was

on chromosome 1B at 49 cM. Within a 6.8cM area, two SNPs (Excalibur_c1841_368 [48.8 cM] and Kukri_c31288_772 [85.57cM]) flanked this A-QTL. The right flanking marker was co-segregated with BobWhite_c1318_691 SNP, while the left flanking marker was a distinct SNP. This A-QTL increased the YPC by 0.133 ppm. On chromosome 2A-1, another minor A-QTL was identified (52 cM). The A-QTL *D1.YPC2A.ndsu* explained 6.7 % PVE with a LOD of 6.4 across locations (Table 3.5). Two SNPs (Excalibur_c59428_270 [51.97 cM] and Tdurum_contig56157_1205 [52.44 cM]) surrounded this A-QTL within a 0.5 cM region. Both flanking markers were unique SNPs. This A-QTL increased YPC by 0.127 ppm. The minor A-QTL *D1.YPC2B.ndsu* was found to be related to YPC on 3 cM on chromosome 2B. This A-QTL was found across locations and explained 5.0 % PVE with a LOD of 4.5. This A-QTL was flanked by two SNPs (Tdurum_contig41920_93 [52.22cM] and Tdurum_contig71139_134 [547.658 cM]). Both flanking markers were unique SNPs. This A-QTL enhanced YPC levels by 0.108 ppm (Table 3.5). The next minor A-QTL (*D1.YPC4A.ndsu*) associated with YPC was found on chromosome 4A-2 at 47 cM. This marker was flanked with two SNPs (Excalibur_c74397_238 [37.45 cM] and Excalibur_c43926_163 [47.53 cM]). This A-QTL explained 4.1% of PVE with LOD of 3.7. This A-QTL had an additive effect of 0.098 ppm on YPC in Langdon location and was not found in Williston location (Table 3.5).

In total for both locations, there were four other minor A-QTL. *D1.YPC2BL.ndsu* and *D1.YPC5AL.ndsu* were found in the Langdon location and *D1.YPC3AW.ndsu* and *D1.YPC7AW.ndsu* were found in the Williston location. Among these minor A-QTL, the additive effect on YPC content was between 0.099 to 0.119 ppm (Table 3.5).

In this population, across locations, 19 DE-QTL were found to be associated with YPC. Of these 19 DE-QTL, 12 had a positive additive by additive effect that is ranging from 0.1 to 0.2 ppm on YPC. The other seven DE-QTL had a negative additive by additive effect of 0.09 to 0.12 ppm on YPC. All minor DE-QTL had LOD of 3 to 4.7 (Table 3.6).

Quantitative Trait Loci for Soluble Brown Pigment

Three different A-QTL on chromosome 5A were found to be associated with SBP in POP1. One A-QTL was identified across locations, and one A-QTL was found on each of the locations (Table 3.5). Quantitative trait locus *D1.SBP5A.ndsu* at 239 cM (found across locations) had a more significant effect on SBP than other A-QTL, with a PVE of 14.9%. With a LOD value of 6.6, the SNPs IWB24494 (left

marker) and Tdurum_contig48766_257 (right marker) flanked this A-QTL within a 2 cM area (238.75 to 240.78 cM on 5A) (Table 3.5). The flanking markers were both unique loci. Across locations, this A-QTL had an additive effect of 0.011 (OD_{400}). In Langdon trials, the A-QTL *D1.SBP5AL.ndsu* at 204 cM on chromosome 5A had 12% PVE with a LOD of 5.2. This A-QTL was flanked with two markers in a 3.2 cM region on chromosome 5A. The left marker was a unique marker (BS00009369_51), while the right flanking marker (wsnp_Ex_c53983_57032473) co-segregated with another marker (Kukri_c38748_584) at 206.1 cM. This A-QTL also had an additive effect of 0.015 (OD_{400}) in Langdon location. In Williston location, the A-QTL *D1.SBP5AW.ndsu* at 144 cM on chromosome 5A had 9.2% PVE with a LOD of 3. In a 6.2 cM area on chromosome 5A, this A-QTL was flanked by two markers. The left marker (tplb0050c03_1003) was unique, but the right marker (Ra_c3966_2205) co-segregated with four other markers (Tdurum_contig81907_126, Tdurum_contig10587_601, Tdurum_contig54543_173, and Tdurum_contig54543_1242). This A-QTL had an additive effect of 0.009 (OD_{400}) in Williston experiments (Table 3.5).

A total of 33 DE-QTL were detected that were associated with SBP. All of them (31) reduced the SBP in this population, and only two added more SBP in the population. The lowest LOD was 3.1, and the highest LOD was 8.2 (Table 3.6).

Quantitative Trait Loci for Polyphenol Oxidase

Four different A-QTL (one from across locations and Three in the Langdon location) were identified to be associated with PPO (Table 3.5). Additive QTL *D1.PPO5A.ndsu* on chromosome 5A at 162 cM position with LOD of 2.5 and PVE of 6.5% was identified. Across locations, this A-QTL has an additive effect of 0.031 (ΔA475/min g). The left flanking marker was Kukri_c49530_762 and was a unique locus, while marker Ku_c12469_983 co-segregated with the other three markers (Ku_c12469_837, Kukri_c59540_137, and IWB69620) in the right side of this A-QTL in a 5.5 cM region. Three other minor effect A-QTL for PPO enzyme activity were detected in Langdon location (Table 3.5). The first A-QTL (*D1.PPO1AL.ndsu*) was detected on chromosome 1A-2 at 105 cM position. This A-QTL, with LOD of 4.3 and PVE of 8.1% had an additive effect of 0.006 (ΔA475/min g) on PPO enzyme activity. The left flanking marker (BobWhite_c46349_402) was a unique locus in 104.84 cM, while the right flanking marker (wsnp_Ku_rep_c71909_71634013) co-segregated at 107.15 cM locus with other seven markers

(wsnp_Ku_rep_c71909_71634013, wsnp_RFL_Contig2808_2585736, Tdurum_contig43360_1592, TA001042-0912, Kukri_c28532_103, Ra_c2322_1067, and BS00031200_51) in a 2.3 cM region (Table 3.5). The second A-QTL in Langdon location is *D1.PPO3AL.ndsu* and was found on chromosome 3A-1 at the 115 cM position. This A-QTL was tightly flanked in the 0.7 cM region, and the left and the right flanking markers were Tdurum_contig12430_156 (114.77 cM) and RAC875_c66892_58 (115.42 cM), respectively. Both flanking markers were unique loci. The A-QTL *D1.PPO3AL.ndsu* had LOD of 4.1 and PVE of 7.8% and added 0.005 (ΔA475/min g) to the Langdon location. The last A-QTL (*D1.PPO4AL.ndsu*) was detected on chromosome 4A-1 at the 56 cM position. This A-QTL with LOD of 2.6 and PVE of 4.8% had an additive effect of 0.004 (ΔA475/min g) in Langdon. This A-QTL was tightly flanked in 0.3 cM regions. Both the left marker (wsnp_Ex_rep_c66426_64645835 in 55.81 cM) and right marker (BobWhite_c31621_148 in 56.08 cM) were unique loci. There were no A-QTL detected in Williston location for this population (Table 3.5).

Nine DE-QTL were detected that were associate with PPO activity. Eight of them decreased the amount of PPO and had a positive additive by the additive effect on SBP. Only one of them increases the amount of PPO in this population (Table 3.6). Some of these DE-QTL had 4% PVE on PPO enzyme activity.

Quantitative Trait Loci for Peroxide

While there were no A-QTL for POD enzyme activity, 91 DE-QTL were detected to be associated with POD enzyme activity. From these 91 DE-QTL in POP1, 67 of them had a positive additive by an additive effect on POD and could decrease POD amount in this population. Two DE-QTL on chromosome 5A, with LOD of 25.3 decreased POD by 3.44 (U/g) in this population (Table 3.6).

Table 3.5. Identified additive-quantitative trait loci (A-QTL) for yellow pigment content (YPC), soluble brown pigment (SBP), polyphenol oxidase (PPO), and peroxide (POD) in population one (Joppa x D121118).

Trait	A-QTL name	Env.	Chr./ L.G.	Left marker	Right marker	Position (cM)	LOD	Additive effect	PVE (%)	Confidence interval
YPC	*D1.YPC1A.ndsu*	A	1A-1	RFL_Contig2831_1012	Tdurum_contig75952_351	86	2.5	0.080	2.7	1.1
YPC	*D1.YPC1B.ndsu*	A, L, W	1B	Excalibur_c1841_368	Kukri_c31288_772	49	6.9	0.133	7.6	6.8
YPC	*D1.YPC2A.ndsu*	A, L, W	2A-1	Excalibur_c59428_270	Tdurum_contig56157_1205	52	6.4	0.127	6.7	0.5
YPC	*D1.YPC2B.ndsu*	A, L, W	2B	Tdurum_contig41920_93	Tdurum_contig71139_134	3	4.5	0.108	5.0	45.4
YPC	*D1.YPC2BL.ndsu*	L	2B	BS00009540_51	Kukri_c40637_223	92	4.0	-0.102	3.8	0.4
YPC	*D1.YPC3AW.ndsu*	W	3A-1	Ra_c8717_520	Tdurum_contig50389_317	53	3.9	0.119	4.7	3.9
YPC	*D1.YPC4A.ndsu*	A, L	4A-2	Excalibur_c74397_238	Excalibur_c43926_163	47	3.7	0.098	4.1	10.1
YPC	*D1.YPC5AL.ndsu*	L	5A	wsnp_Ex_rep_c70117_69067356	Tdurum_contig54785_62	266	3.6	-0.096	3.5	2.3
YPC	*D1.YPC5B.ndsu*	A, L, W	5B-2	BS00064947_51	wsnp_Ra_c12489_9996904	0	16.0	0.214	16.0	2.2
YPC	*D1.YPC6B-2.ndsu*	L, W	6B-2	IWB65555	IWB10806	17	6.8	0.161	8.9	18.1
YPC	*D1.YPC6B.ndsu*	A	6B-2	IWB10806	IWB9108	27	7.2	0.170	11.6	18.3
YPC	*D1.YPC7AW.ndsu*	W	7A-1	Tdurum_contig17697_675	RAC875_c13300_139	35	2.9	-0.099	3.5	4.2
SBP	*D1.SBP5AW.ndsu*	W	5A	tplb0050c03_1003	Ra_c3966_2205	144	3.0	-0.009	9.2	6.2
SBP	*D1.SBP5AL.ndsu*	L	5A	BS00009369_51	wsnp_Ex_c53983_57032473	204	5.2	-0.015	12.0	3.2
SBP	*D1.SBP5A.ndsu*	A	5A	IWB24494	Tdurum_contig48766_257	239	6.6	-0.011	14.9	2.0
PPO	*D1.PPO1AL.ndsu*	L	1A-2	BobWhite_c46349_402	wsnp_Ku_rep_c71909_71634013	105	4.3	0.006	8.1	2.3
PPO	*D1.PPO3AL.ndsu*	L	3A-1	Tdurum_contig12430_156	RAC875_c66892_58	115	4.1	0.005	7.8	0.7
PPO	*D1.PPO4AL.ndsu*	L	4A-1	wsnp_Ex_rep_c66426_64645835	BobWhite_c31621_148	56	2.6	-0.004	4.8	0.3
PPO	*D1.PPO5A.ndsu*	A	5A	Kukri_c49530_762	Ku_c12469_983	162	2.5	0.031	6.5	5.5

* Environment (Env.), Chromosome (Chr.), linkage group (L.G.), phenotypic variation (PVE), across locations (A), Langdon (L), and Williston (W).

Table 3.6. Summary of identified digenic epistatic-quantitative trait loci (DE-QTL) for yellow pigment content (YPC), soluble brown pigment (SBP), polyphenol oxidase (PPO), and peroxide (POD) in population one (Joppa x D121118).

DE-QTL Name	Chr. Name	Pos. (cM)	Left marker	Right marker	Ass. Traits	Ass. A-QTL	LOD	PVE (%)	Add. by Add.
----------Yellow pigment content----------									
D1E.YPC5B-7A.ndsu	5B-2 7A-1	0 20	BS00064947-51 Tdurum_contig59908_400	wsnp_Ra_c12489_19996904 BS00093896_51	*	YPC	3.1	13.6	0.100
D1E.YPC1B-5B.ndsu	1B 5B-3	20 0	Kukri_c79135_136 Tdurum_contig98215_420	Tdurum_contig46780_203 Tdurum_contig81337_335	*	*	4.4	6.8	0.200
----------Soluble brown pigment----------									
D1E.SBP3A-3A.ndsu	3A-1 3A-1	155 160	IWB29794 IWB29794	Tdurum_contig75764_1038 Tdurum_contig75764_1038	POD	*	7.3	2.7	-0.022
D1E.SBP2B-2B.ndsu	2B 2B	45 50	Tdurum_contig41920_93 Tdurum_contig71139_134	Tdurum_contig71139_134 RAC875_c4314_993	*	YPC	6.9	2.4	-0.033
D1E.SBP2A-2B.ndsu	2A-1 2B	30 35	Kukri_c84087_154 Tdurum_contig41920_93	BS00016676_51 Tdurum_contig71139_134	POD	YPC	4.3	2.4	-0.016
----------Polyphenol oxidase----------									
D1E.PPO1B-4A.ndsu	1B 4A-1	90 30	D_contig17842_656 Kukri_c2963_272	Tdurum_contig11130_90 Tdurum_contig13606_928	POD	*	4.4	4.1	-0.062
D1E.PPO4A-4A.ndsu	4A-1 4A-1	45 50	Kukri_c2963_272 Kukri_c2963_272	Tdurum_contig13606_928 Tdurum_contig13606_928	SBP, POD	*	3.6	4.0	-0.109
----------Peroxide----------									
D1E.POD5A-5A.ndsu	5A 5A	105 110	CAP12_c2984_189 CAP12_c2984_189	BS00041911_51 BS00041911_51	*	*	25.3	0.6	-3.439
D1E.POD2A-2A.1.ndsu	2A-1 2A-1	20 25	Kukri_c84087_154 Kukri_c84087_154	BS00016676_51 BS00016676_51	SBP	*	24.3	0.6	-3.777
D1E.POD4A-4A.1.ndsu	4A-1 4A-1	25 30	Kukri_c2963_272 Kukri_c2963_272	Tdurum_contig13606_928 Tdurum_contig13606_928	SBP, PPO	*	18.7	0.5	-1.736
D1E.POD2A-2B.ndsu	2A-1 2B	30 25	Kukri_c84087_154 Tdurum_contig41920_93	BS00016676_51 Tdurum_contig71139_134	SBP	YPC	15.7	0.5	-1.833

Population Two (Mountrail x Carpio)

Quantitative Trait Loci for Yellow Pigment Content

QTL mapping identified one major and stable A-QTL and two minor A-QTL associated with YPC. Another small A-QTL was discovered to be associated with the YPC in Langdon location (Table 3.7). *D2.YPC7A.ndsu* on chromosome 7A-1, with a PVE of 29.2 %, showed a more significant effect on YPC than other A-QTL. With a LOD score of 23, the SNPs Tdurum_contig45062_417 (left marker) and Tdurum_contig4676_3675 (right marker) tightly flanked this A-QTL within a 0.4 cM region (48.92 to 49.36 cM). Both flanking markers are unique loci (Table 3.7). The additive effect of this A-QTL on YPC was 0.52 ppm in Langdon, 0.56 ppm in Williston, and 0.567 ppm across locations. The *D2.YPC5B.ndsu* A-QTL on chromosome 5B-1 was found at 78 cM and explained 3.5% of phenotypic variation in YPC with a LOD of 3.5. Within a 2.2 cM area, this A-QTL was flanked by two SNPs (CAP8_rep_c5825_165 [85.41 cM] and Excalibur_c74858_243 [86.53 cM]) (Table 3.7). The right flanking marker shared a locus with another SNP (BS00003944_51); however, the left flanking marker is a distinct locus. This A-QTL had an additive effect of 0.2 ppm on YPC across location. The second minor A-QTL linked to YPC was discovered on chromosome 7B-1 at 90 cM. Two SNPs, CAP7_c122_102 and IAAV1369, flanked this marker, with positions of 89.31 and 90.26 cM, respectively, within a 1.0 cM region. With a LOD of 3.7, the A-QTL *D2.YPC7B.ndsu* explained 3.8 % of PVE. In addition, this A-QTL had a 0.3 ppm additive effect on YPC in Williston location and was not detected in Langdon location. The left flanking marker was found to be co-segregating with additional SNPs (wsnp_Ex_c27914_37075168 and Tdurum_contig28644_90). However, the right flanking marker was found to be a single SNP. Also, in Langdon, another A-QTL on chromosome 7B-1 was identified to be associated with YPC at 67 cM with a PVE of 4% and LOD of 3.5 (Table 3.7). The *D1.YPC7BL.ndsu* A-QTL was linked with two flanking markers (Tdurum_contig75644_138 and CAP7_c10566_170) within a 3.6 cM area. Both flanking markers co-segregated with other markers. The left flanking marker co-segregated with Tdurum_contig75644_871 at 63.46 cM, and the right flanking marker co-segregated with six more SNPs (BS00079019_51, IAAV7544, Tdurum_contig84962_266, BobWhite_rep_c64768_264, IAAV3391, and Tdurum_contig84962_256) at 67.04 cM (Table 3.7).

In this population, only four DE-QTL were detected for YPC. Three of them had a negative additive by the additive effect of 1.9 ppm on YPC. The DE-QTL detected on chromosome 7B had a

positive additive by the additive effect of 0.28 ppm on YPC. The LOD for all four DE-QTL in this population was from 3 to 3.6 (Table 3.8).

Quantitative Trait Loci for Soluble Brown Pigment

Only two minor A-QTL were found to be associated with SBP. One of the A-QTL was detected across locations and the other only in the Williston location (Table 3.7). The A-QTL *D2.SBP1B.ndsu* was detected on chromosome 1B-1 at the 125 cM position. This marker is flanked by Tdurum_contig52053_149 (119.43 cM) and GENE-0549_18 (127.51 cM) in the 8.1 cM region. Both markers are unique loci. The additive effect for this marker in Williston location was 0.021 (OD_{400}), and across locations was 0.012 (OD_{400}). Table 3.7 shows all the A-QTL for SBP. In Langdon trails, the A-QTL *D2.SBP4AL.ndsu* with PVE of 7% and LOD of 3 was associated with SBP. This A-QTL was detected on chromosome 4A at 102 cM. Two flanked markers are tightly linked with this A-QTL, the left marker is wsnp_Ku_c4924_8816643 at 101.31 cM, and the right marker is Tdurum_contig47148_651 at 102.22 cM. This A-QTL added 0.006 (OD_{400}) to Langdon trials.

For DE-QTL, a total of 17 DE-QTL were found to be associate with SBP. All of them had a positive additive by additive effect between 0.02 to 0.06 (OD_{400}) on SBP and decreased the total SBP in POP2. The lowest LOD was 3.2, while the highest LOD among these DE-QTL was 12.2 (Table 3.8).

Quantitative Trait Loci for Polyphenol Oxidase

One primary and stable A-QTL was found that was associated with PPO enzyme activity. Additive-QTL *D2.PPO2A.ndsu* was detected across locations and on chromosome 2A-3 at the 13 cM position. This A-QTL had a LOD of 8.5 and explained 19.1% effect on phenotypic variation. In a region of 7.2 cM, two markers co-segregated and flanked this A-QTL (Table 3.7). The left marker was IACX5800 and co-segregated with the marker BS00003663_51 over a 6.42 cM region. The right flaking marker, which was closer to the A-QTL, was RAC875_rep_c69619_78 and co-segregated the 13.57cM locus with wsnp_Ex_c41007_47932833, wsnp_CAP11_c1711_934478, and RAC875_c104160_61 markers. This A-QTL had an additive effect of 0.41 (ΔA475/min g) on PPO in Langdon location and only an additive effect of 0.04 (ΔA475/min g) in Williston location (Table 3.7). Across locations, this A-QTL had an additive effect of 0.034 (ΔA475/min g) on PPO. The other two minor A-QTL were detected on chromosomes 3A-2 and 7A-1 at 67 and 44 cM, respectively. Additive QTL *D2.PPO3A.ndsu* with LOD of 4.8 and PVE of 9.9% had

a large additive effect of 1.264 (ΔA475/min g) on PPO. This A-QTL was tightly flanked in a 0.7 cM region with two markers. The left marker in the 66.52 cM position was RAC875_c61934_406, and the right marker in the 67.49 cM position was Tdurum_contig100787_79. Both markers were unique markers. The second A-QTL was *D2.PPO7A.ndsu* was flanked with Tdurum_contig59908_400 (left marker) and Tdurum_contig98197_73 (right marker) in a 6.4 cM region (Table 3.7). The left marker at 38.51 cM was a unique locus, but the right marker at 44.9 cM co-segregated with marker Tdurum_contig24133_438. This A-QTL, with LOD of 3 and PVE of 6.2% had an additive effect of 0.991 (ΔA475/min g) on PPO enzyme activity.

Ten DE-QTL were found for PPO enzyme activity. Out of ten, six of them decreased 0.07 (ΔA475/min g) PPO activity, and four of them increased 0.02 to 0.07 (ΔA475/min g) PPO enzyme activity in this population. The lowest LOD for these DE-QTL was 3.4, and the highest LOD was 7.2 (Table 3.8).

Quantitative Trait Loci for Peroxidase

In POP2 and only in Williston were one major and two minor A-QTL associated with POD enzyme activity. The major A-QTL *D2.POD3AW.ndsu* was detected on chromosome 3A-2 at 75 cM position with a LOD of 35.3 and PVE of 53.3% (Table 3.7). In Williston trials, this A-QTL had an additive effect of 1.24 (U/g) on POD activity. This A-QTL was tightly flanked in a 0.6 cM region with two unique markers at the 74.57 and 75.13 cM (markers RAC875_c63035_147 and kukri_rep_c92293_249, respectively). The other two minor A-QTL were detected on chromosomes 1A-2 and 4A. The A-QTL on chromosome 1A-2 (*D2.POD1AW.ndsu*) at the 32 cM position had a LOD of 3.2 and PVE of 3.2%. This A-QTL was flanked with Tdurum_contig5008_556 as the left marker and RAC875_c65431_351 as the right marker in a 1.3 cM region (Table 3.7). Both markers were unique loci. This A-QTL had an additive effect of 0.3 (U/g) on POD in the Williston location. The second minor A-QTL (*D2.POD4AW.ndsu*) was detected on 66 cM position on chromosome 4A with LOD of 3.4 and PVE of 3.4%. The left flanked marker (GENE-4933_1085) was closer to this A-QTL in 65.64 cM, while the right flanked marker (IACX2890) was far from this A-QTL in 91.1 cM. Both flanking markers were unique loci (Table 3.7). This A-QTL had an additive effect of 0.309 (U/g) on POD in the Williston location.

Only 20 DE-QTL were detected for POD activity (Table 3.8). The lowest LOD was 3.2, and the highest LOD was 34.2. Two DE-QTL on chromosome 2B-1 decreased POD by 8.2 (U/g) in this

Population. From these 20 DE-QTL, 19 of them had a positive additive by the additive effect on POD that reduced the amount of POD in this population.

Table 3.7. Identified additive-quantitative trait loci (A-QTL) for yellow pigment content (YPC), soluble brown pigment (SBP), polyphenol oxidase (PPO), and peroxide (POD) in population two (Mountrail x Carpio).

Trait	A-QTL name	Env.	Chr./ L.G.	Left marker	Right marker	Position (cM)	LOD	Additive effect	PVE (%)	Confidence interval
YPC	*D2.YPC5B.ndsu*	A, L, W	5B-1	CAP8_rep_c5825_165	Excalibur_c74858_243	78	3.5	-0.200	3.5	2.2
YPC	*D2.YPC7A.ndsu*	A, L, W	7A-1	Tdurum_contig45062_417	Tdurum_contig4676_3675	49	23	-0.567	29.2	0.4
YPC	*D2.YPC7BL.ndsu*	L	7B-1	Tdurum_contig75644_138	CAP7_c10566_170	67	3.5	-0.181	4.0	3.6
YPC	*D2.YPC7B.ndsu*	A, W	7B-1	CAP7_c122_102	IAAV1369	90	3.7	-0.204	3.8	1.0
SBP	*D2.SBP1B.ndsu*	A, W	1B-1	Tdurum_contig52053_149	GENE-0549_18	125	3.7	0.012	8.5	8.1
SBP	*D2.SBP4AL.ndsu*	L	4A	wsnp_Ku_c4924_8816643	Tdurum_contig47148_651	102	3.0	-0.006	7.0	0.9
PPO	*D2.PPO2A.ndsu*	A, L, W	2A-3	IACX5800	RAC875_rep_c69619_78	13	8.5	-0.034	19.1	7.2
PPO	*D2.PPO3A.ndsu*	A	3A-2	RAC875_c61934_406	Tdurum_contig100787_79	67	4.8	-1.264	9.9	0.7
PPO	*D2.PPO7A.ndsu*	A	7A-1	Tdurum_contig59908_400	Tdurum_contig98197_73	44	3.0	-0.991	6.2	6.4
POD	*D2.POD1AW.ndsu*	W	1A-2	Tdurum_contig5008_556	RAC875_c65431_351	32	3.2	0.300	3.2	1.3
POD	*D2.POD3AW.ndsu*	W	3A-2	RAC875_c63035_147	Kukri_rep_c92293_249	75	35.3	-1.240	53.3	0.6
POD	*D2.POD4AW.ndsu*	W	4A	GENE-4933_1085	IACX2890	66	3.4	-0.309	3.4	25.4

* Environment (Env.), Chromosome (Chr.), linkage group (L.G.), phenotypic variation (PVE), across locations (A), Langdon (L), and Williston (W).

Table 3.8. Summary of identified digenic epistatic-quantitative trait loci (DE-QTL) for yellow pigment content (YPC), soluble brown pigment (SBP), polyphenol oxidase (PPO), and peroxide (POD) in population two (Mountrail x Carpio).

DE-QTL Name	Chr. Name	Pos. (cM)	Left marker	Right marker	Ass. Traits	Ass. A-QTL	LOD	PVE (%)	Add. by Add.
----------Yellow pigment content----------									
D2E.YPC7B-7B.ndsu	7B-1 7B-2	110 10	RAC875_rep_c73990_174 RAC875_c527_106	Kukri_c50071_1084 Kukri_c18148_913	*	*	3.0	13.6	0.300
D2E.YPC1B-3A.ndsu	1B-1 3A-2	15 90	BS00105846_51 wsnp_Ex_c45877_51547406	Excalibur_c10065_570 wsnp_Ex_c45877_51548342	*	*	3.6	7.6	-0.200
D2E.YPC5B-7A.ndsu	5B-3 7A-2	0 0	Tdurum_contig44115_561 BS00060187_51	BS00022886_51 wsnp_Ex_c6961_12000176	*	*	3.6	6.3	-0.200
D2E.YPC1A-5B.ndsu	1A-2 5B-3	60 35	wsnp_Ex_c4605_8239915 Tdurum_contig12776_278	BS00038929_51 RAC875_c28645_455	*	*	6.2	6.2	-0.200
----------Soluble brown pigment----------									
D2E.SBP2A-2A.ndsu	2A-1 2A-1	45 50	Tdurum_contig26621_200 Tdurum_contig60235_622	Kukri_c102346_668 Tdurum_contig15438_231	POD	*	12.2	1.7	-0.056
D2E.SBP2B-2B.1.ndsu	2B-1 2B-1	10 15	Tdurum_contig99313_288 Tdurum_contig99313_288	Tdurum_contig25602_212 Tdurum_contig25602_212	POD	*	11.3	1.7	-0.031
D2E.SBP2B-2B.2.ndsu	2B-2 2B-2	10 10	IWB32293 IWB32293	BS00009060_51 BS00009060_51	POD	*	8.1	1.7	-0.024
----------Polyphenol oxidase----------									
D2E.PPO2A-2A.ndsu	2A-1 2A-1	20 80	Tdurum_contig26621_200 BS00067499_51	Kukri_c102346_668 Excalibur_c9206_836	*	*	7.2	3.4	0.067
D2E.PPO4A-4B.ndsu	4A 4B	45 70	BS00040668_51 RAC875_c6865_349	Tdurum_contig46983_325 Tdurum_contig8322_2041	*	*	6.5	3.4	-0.068
D2E.PPO4B-5B.ndsu	4B 5B-3	75 15	RAC875_c6865_349 BobWhite_c38408_71	Tdurum_contig8322_2041 BS00064947_51	*	*	3.9	3.3	0.067
----------Peroxide----------									
D2E.POD5B-5B.1.ndsu	5B-1 5B-1	35 40	Kukri_c51_98 Kukri_c51_98	BS00064042_51 BS00064042_51	SBP	*	34.2	2.2	-5.889
D2E.POD1A-1A.ndsu	1A-2 1A-2	25 45	RAC875_c56994_301 Tdurum_contig69753_513	IAAV5652 IACX2325	*	*	30.6	2.1	-5.922
D2E.POD2B-2B.ndsu	2B-1 2B-1	5 10	wsnp_Ex_c45094_50985067 Tdurum_contig99313_288	GENE-2904_476 Tdurum_contig25602_212	SBP	*	31.9	1.9	-8.215

Discussion

The QTL mapping study found discrepancies in the quantity and map position of QTL found in various analyses (Kumar et al., 2016; Oladzad-Abbasabadi et al., 2018; Kumar et al., 2018; Rabbi et al., 2021). This could be related to the different plant material used in each study and the analytical and statistical processes used (e.g., linkage mapping vs. GWAS). Furthermore, the trait measurement approach and statistical procedures used may influence the reliability of each QTL analysis. Indeed, the presence of several genes with additive effects on the traits, parental influence on the genotypes of mapping populations, genotype-environment interaction, and the number of markers utilized may all have an impact on the results (Naraghi et al., 2019).

Genetics of Yellow Pigment Content

In line with the production of new durum cultivars, many breeding projects worldwide focus on developing high-quality traits such as color (Battenfield et al., 2016). Yellow pigment content is the essential component in determining the color of pasta (Colasuonno et al., 2019). Previous research has shown a few major and several minor QTL for YPC, indicating this trait has a polygenetic nature and quantitative inheritance, similar to our findings (Elouafi et al., 2001; Mares and Campbell, 2001; Hessler et al., 2002; Li-Ping et al., 2006; Pozniak et al., 2007; Dubcovsky, 2008; He et al., 2008; Patil et al., 2008; Howitt et al., 2009; Singh et al., 2009; Zhang et al., 2009; Blanco et al., 2011) reported the quantitative nature of this trait.

As Ficco et al. (2014) reported, several major QTL on chromosome 7 are associated with YPC. In our study, on chromosome 7A, the most significant A-QTL (*D2.YPC7A.ndsu*) was identified. This A-QTL had a PVE of 32.7% and 18.9% in the Langdon and Williston trials, respectively. Also, this A-QTL was detected across both locations and had a PVE of 30%. This A-QTL supports the research conducted by Johnson et al. (2019), in which they found the QTL on chromosome 7A that is related to YPC and associated with Color_b and Dif_b trait in durum wheat. However, in previous research, none of the flanking markers are characterized, and to our knowledge this is the first time they have been reported.

Previous research (Borreli et al., 1999; Ficco et al., 2014; Colasuonno et al., 2019) indicated that YPC was controlled more by genotype than the environment. In Pop1, *D1.YPC5B.ndsu* was found in both locations Langdon and Williston indicating no genotype and environment interaction affect YPC and

indicates YPC is mainly controlled by genotype rather than environment. The A-QTL *D2.YPC7A.ndsu* came from Carpio, which is one of the best cultivars in the NDSU breeding program for having high yellow color (Elias et al., 2015). Similarly, this A-QTL was found in both locations indicating no genotype by environment interaction.

Our results support the Colasunno et al. (2019) study which found two new QTL related to YPC in chromosome 5B in their research. In the current study in POP1, another major A-QTL (*D1.YPC5B.ndsu*) was detected on chromosome 5B, which explained 20% of the YPC trait phenotypic variation. However, in POP2, a major QTL *D2.YPC7A.ndsu* was found on chromosome 7A which explained 29% of the variation and only two minor QTL were found on chromosomes 5B and 7B.

Another significant and stable A-QTL was detected on chromosome 6B in POP1 and had a PVE of 11.5% on YPC. This A-QTL (*D1.YPC6B.ndsu*) came from Joppa and had a large effect on increasing YPC in this population. Other studies suggested that one QTL on chromosome 6B is related to YPC and YI (Pozniak et al., 2007; Colasunno et al., 2019; Johnson et al., 2019). The right marker of this QTL (IWB9108) also affects thousand kernel weight and the number of spikes in winter wheat (Assanga et al., 2017). These results indicate this marker plays a significant role in YPC and yield-related wheat traits.

Minor A-QTL play a significant role in measuring heritability and enhancing prediction accuracy in breeding programs, according to Uemoto et al. (2015). The discovery of such effects may help us understand the relative disparities in the range of color-related phenotypes in various contexts. Individuals in the Langdon trials, for example, consistently had a higher YPC than those in the Williston trials. Some environmental factors may influence this conclusion; however, the minor A-QTL in Langdon explains more than 40% of PVE on YPC, while the total minor A-QTL effect in Williston is slightly more than 30%. This finding demonstrated the significance of small A-QTL in the YPC trait. In previous research minor A-QTL have been found on chromosomes 1A, 1B, 2A, 2B, 4A, 5B, and 7B, similar to our results (Pozniak et al., 2007; Zhang and Dubcovsky, 2008; Johnson et al., 2019). Also, a PVE of less than five percent for YPC was identified in other A-QTL (on chromosomes 1A, 2B, 3A, 4A, 5A, 5B, 7A, and 7B). The right flanking marker (Excalibur_c74858_243) in a minor QTL on chromosome 5B (*D2.YPC5B.ndsu*) also affects growing stages in wheat (Kronenberg et al., 2019). In their study, stem elongation was measured by two growing degree-days (15% and 95%) and both growing degrees were significantly associated with

Excalibur_c74858_243. These results show that this maker can be used to select for both YPC and stem elongation in wheat.

The right flanking marker (CAP7_c10566_170) in the minor QTL (*D2.YPC7BL.ndsu*) could control pre-harvest sprouting in wheat. This marker on chromosome 7B is also known as the candidate gene for pre-harvest sprouting in purple false brome (*Brachypodium distachyon*) and rice (Cabrel et al., 2014). These results show how effective this marker could be in selecting for several important traits in MAS.

Some isoforms of *the PSY* gene in chromosomes 3 and 5 in durum wheat control YPC accumulation in kernels (Dibari et al., 2012). Also, previous research showed the alternate allele of *the PSY-A1* gene was associated with higher yellow color in durum wheat (Campos et al., 2016; Vargas et al., 2016). Dong et al. (2012) found *the ZDS* gene on chromosome 2A explained 11.3% of the PVE of YPC in their study. Future fine-mapping studies are needed to compare the minor A-QTL on chromosome 2A (*D1.YPC2A.ndsu*) and previously known gene on chromosome 2A for *ZDS* gene.

Epistatic interactions have been shown to have an impact on traits in durum wheat (Kulwal et al., 2005; Zhao et al., 2010). Along with all these major and minor A-QTL, many DE-QTL were identified to be associated with YPC in both populations and could increase or decrease the amount of YPC. Roncallo et al. (2012) reported major DE-QTL on chromosomes 7A and 7B that affect YPC. One of the important pairs of DE-QTL in the current study in POP2 was on chromosome 7B that increased the amount of YPC in this population by 0.28 ppm. Another minor DE-QTL on chromosome 2B (*D1.YPC2B.ndsu*) with a PVE of 5% was found to be associated with cadmium uptake in durum wheat (Oladzad-Abbasabadi et al., 2018). The right flanking marker for this DE-QTL (Tdurum_contig71139_134) has a positive epistatic effect on cadmium uptake in durum wheat. Another minor DE-QTL was detected on chromosome 6B, which had an additive effect of 8.9% on YPC. Neither of these flanking markers for this DE-QTL were reported in previous studies. The results in the current study show the DE-QTL in POP1 had a negative PVE of 22% on YPC and decreased YPC by 0.74 ppm. On the other hand, the positive DE-QTL in POP1 had a PVE of 42% and increased YPC by 1.52 ppm. These results indicate that in the absence of the important genes on chromosome 5B, the epistatic interaction can control the amount of YPC. However, in POP2, with a major A-QTL (*D2.YPC7A.ndsu*), epistatic interactions had proportionately less of an effect on YPC.

Our results in this chapter support the results of chapter two, in which the cultivar Mountrail and experimental line D121118 had the lowest YPC among checks. This result indicates three major genes and almost all of the minor A-QTL associated with YPC came from the other two parents of these populations (Carpio and Joppa). Our results indicate Joppa (with two major A-QTL, which explained more than 30% of PVE) could be a good source for a high YPC in the NDSU breeding program.

Genetics of Soluble Brown Pigment

Pasta color is essentially a combination of yellowness and brownness (Kobrehel et al., 1974), with the addition of a red coloration that occurs under particular drying circumstances (Feillet and Dexter, 1996). Pasta's brightness and yellowness are essential quality factors for pasta manufacturers and customers (Troccoli et al., 2000). According to research, there are two sources of pasta brownness: enzymatic and non-enzymatic sources (Matsuo and Irvine, 1967; Kobrehel et al., 1974). A water-soluble brown-cupric protein (Matsuo and Irvine, 1967) or enzymes such as PPO and POD can cause a genetically controlled brownness (Kobrehel et al., 1974). Brown pigments are well-known for their ability to dim the brightness of yellow tones (Matsuo and Irvine 1967; Feillet et al., 2000). Soluble brown pigments from the Maillard reaction during processing and semolina imperfections could be responsible for pasta's brown color (Feillet et al., 2000). Previous studies measured the effect of the PPO enzyme on the brown color of pasta during pasta processing (Kobrehel et al., 1974; Demeke et al., 2001; Verlotta et al., 2010; Colasuonno et al., 2019). However, none of them looked for genes responsible for SBP in durum wheat. Compared to earlier QTL mapping studies, and based on our knowledge, this is the first time to report the use of a 90K SNPs assay for dissection of SBP in durum wheat. A total of five A-QTL (three in POP1 and two in POP2) and 50 minor DE-QTL associated with SBP were detected in this study.

Three A-QTL in POP1 for SBP were detected on chromosome 5A in different positions (*D1.SBP5A.ndsu*, D1.SBP5AW.ndsu, and D1.SBP.5AL.ndsu with the PVE of 6.4%, 9.2%, and 12%, respectively). The right flanking marker (Ra_c3966_2205) of *D1.SBP5AW.ndsu* also affects flowering time in spring wheat (Zou et al., 2017). This marker might be important in durum wheat breeding programs in selecting for SBP and flowering time.

As we indicated earlier Carpio and Joppa have high YPC. All SBP A-QTL came from the experimental line D121118 and Mountrail, which indicates that SBP is commonly available in the absence

of YPC QTL. As a result, line D121118 has a dimmer color than Joppa, and Joppa has a high yellow color because of the absence of SBP QTL. Also, the expression of YPC in Joppa was not diminished by the effects of deleterious alleles for SBP. Among the top five low SBP progenies, three have the QTL, which increased the amount of SBP and showed the importance of these QTL when selecting for low brown color pasta.

In POP1, the epistatic effect had an important role in controlling SBP. Thirty-three DE-QTL were detected in POP1. Thirty-one of 33 pairs reduced the amount of SBP by 0.61 (OD_{400}), which indicates the significant roles of DE-QTL on SBP. Each DE-QTL in POP1 was a pair between a QTL from Joppa and one QTL derived from experimental line D121118. The DE-QTL have a positive effect on SBP and reduced the amount of SBP in the population. These results indicate that crossing Joppa and experimental line D121118 could lead to having DE-QTL, which contribute to reductions in SBP in the population. The high LOD for these DE-QTL explains the effectiveness of these DE-QTL on SBP. Only two DE-QTL (when both pairs of QTL came from Joppa) had a positive additive by additive effect; when DE-QTL came from D121118 it had a negative effect on SBP.

In POP2, A-QTL on chromosomes 1B and 4A were associated with SBP. The QTL *D2.SBP1B.ndsu* with a PVE of 8.5% on SBP came from Mountrail and could be related to the lower yellowness of this cultivar. The left flanking marker (Tdurum_contig52053_149) of this A-QTL also affects grain protein concentration (GPC) in Canadian durum wheat. Ruan et al. (2021) reported that this marker on chromosome 1B decreased GPC in durum wheat. Selecting against this marker could increase yellowness and GPC in durum wheat.

The A-QTL *D2.SBP4AL.ndsu* with a PVE of 7% came from Carpio. Although this A-QTL could increase SBP, the major A-QTL on chromosome 7A (*D2.YPC7A.ndsu*) that provide high YPC might mask SBP and increase the total amount of YPC in a population. It was reported previously by Naraghi et al. (2019) that the right flanking marker (Tdurum_contig47148_651) of *D2.SBP4AL.ndsu* has an impact on Mixograph MID line peak time and shows the importance of this area in quality traits in wheat-related species.

In POP2, a total of 17 DE-QTL were found to be associated with SBP. All of the DE-QTL had a positive additive by the additive effect on SBP and reduced the amount of SBP in POP2 by 0.6 (OD_{400}).

Similarly, in POP1 when QTL paired with other parents' QTL, they had a positive effect. Our results show the importance of epistatic effect when there are few A-QTL in the population. Previous research also explained the importance of epistatic QTL on complex traits such as quality traits (Cao et al., 2001; Ma et al., 2005, 2007).

Cabas-Lühmann and Manthey (2020) showed SBP is a complex trait, and both environment and genotype could control this trait. Our results suggest breeding out the A-QTL for SBP could develop high yellow-colored durum varieties. For SBP (like YPC), Carpio and Joppa could be used as two reliable sources in the NDSU durum wheat breeding program to develop durum varieties with good color.

Genetics of Polyphenol Oxidases Activity

Carotenoid pigment degradation is affected by enzymes such as PPO. Several studies have revealed a relationship between PPO activities in durum wheat and the color of the pasta (Matsuo and Irvine, 1967; Kobrehel and Gautier, 1974; Kobrehel et al., 1974; Baik et al., 1994). The final color of pasta is determined by the amount of YPC, SBP, and the activities of the enzymes that affect pigment concentration, such as PPO (Borreli et al., 1999). Polyphenol oxidase is one of the oxidative enzymes that produces SBP in pasta processing (Kobrehel et al., 1974). Polyphenol oxidase catalyzes the oxidation of phenolic acids, resulting in the formation of short-chain polymers that cause unwanted browning or darkening of pasta products, lowering their apparent quality (Watanabe et al., 2006).

The level of PPO activity was found to be more closely associated with growing environment than with the genotype (Park et al., 1997). However, previous studies showed a major QTL on the long arm of chromosome 2A that is linked to PPO activity (Jimenez and Dubcovsky, 1999; Nair and Tomar, 2001; Simeone et al., 2002; Sun et al., 2005; Zhang et al., 2005; Anderson et al., 2006; Watanabe et al., 2006; He et al., 2007; Raman et al., 2009; Sadeque and Turner, 2010; Si et al., 2012). This PPO QTL, with a PVE of 50%, was discovered on chromosomal arm 2AL, 1.4 cM apart, between PPO-A1 and RAC875 c9845 762 (Zhai et al., 2016). Other paralogous PPO genes (PPO-A2 and PPO-B2) have been discovered in group 2 (Beecher and Skinner, 2011; Beecher et al., 2012). Watanabe et al. (2006) showed this QTL on chromosome 2A explained more than 49% of PVE. In POP2, the current study detected a stable and major A-QTL on chromosome 2A *(D2.PPO2A.ndsu*), explaining 19.2% PVE of PPO enzyme activity. The right flanking marker (RAC875_rep_c69619_78) of *D2.PPO2A.ndsu* was associated with a

tify domain-containing protein. Zhang et al. (2019, 2022) reported the tify domain is important in vernalization. These results indicated the importance of chromosome 2A and this specific area on wheat.

Two other minor A-QTL in POP2 (*D2.PPO3A.ndsu* and *D2.PPO7A.ndsu*) were identified that explained 10% and 6.2% of PVE, respectively. These A-QTL were also found in a study by Johnson et al. (2019) in the NDSU durum wheat breeding program. Qaseem et al. (2019) detected the right flanking marker (Tdurum_contig100787_79) in *D2.PPO3A.ndsu* to be associated with biomass and spike length in wheat under stress field conditions.

The non-desirable alleles for PPO in POP2 were all derived from the Carpio cultivar, which was the source of the favorable alleles for YPC. Carpio is well known for high YPC and PPO enzyme activity and is the source for A-QTL related to these traits. However, the major A-QTL on chromosome 7A could mask the QTL responsible for PPO, the same as QTL that control SBP. Thus, the high amount of PPO does not affect the final yellow color in the Carpio cultivar. Mountrail is low in PPO activity; however, it has the A-QTL for SBP, which could dim the yellow color in pasta. The minor A-QTL for PPO on chromosome 7A appears to be novel, and it is possible that it can be related to the various sources of germplasm employed in this study.

In POP1, both parents had a low amount of PPO. Thus, the current study provided a unique population that did not reveal the previously reported QTL for PPO on chromosome 2A in durum wheat (Watanabe et al., 2006). Four minor A-QTL were detected in POP1 on chromosomes 5A (*D1.PPO5A.ndsu* with 6.5% of PVE), 1A (*D1.PPO1AL.ndsu* with 8.1% of PVE), 3A (*D1.PPO3AL.ndsu* with 7.8% of PVE), and 4A (*D1.PPO4AL.ndsu* with 4.8% of PVE). Almost all of these minor A-QTL were derived from Joppa (1A, 3A, and 5A) and one came from experimental line D121118 (4A), but neither of them had a major effect on the phenotype. The low yellow color of D121118 is not caused by PPO enzyme activity; it could be because of the appearance of A-QTL for SBP in this line. Joppa does not have A-QTL for PPO, resulting in high yellow and low brown color for Joppa.

There appear to be two minor A-QTL for PPO on chromosomes 4A and 5A that are unique to this study, and they are probably related to the diverse sources of germplasm used in the research. He et al. (2016) reported the right flanking marker (Ku_c12469_983) of *D1.PPO5A.ndsu* on chromosome 5A has a minor effect on Fusarium head blight (FHB) resistance.

In POP1, there were nine DE-QTL associated with PPO enzyme activity. Most of them could decrease the amount of PPO in POP1 (0.66 [ΔA475/min g]); however, the low LOD (ranging from 3.3 to 5) showed these DE-QTL had less significance on PPO. When both parents are low in PPO, the epistatic effect of pairing QTL from both parents leads to reduced PPO in this population. In POP2, on the other hand, due to having a parent with high PPO (Carpio), four DE-QTL were detected to increase the amount of PPO in the population by 0.22 ΔA475/min g. These results indicated that having one parent with high PPO can increase the amount of PPO, even with the effect of minor DE-QTL.

Genetics of Peroxidase Activity

Peroxidase is one of the enzymes that control the brown color in pasta products. It was shown that pasta manufactured from durum cultivars with high POD activity in the endosperm had a brownish color and a positive relationship between the brown index of pasta and POD activity (Kobrehel et al., 1974; Fraignier et al., 2000). Peroxidase does not appear to be active during the pasta processing, which is most likely due to a lack of available hydrogen peroxide (the enzyme's primary substrate), whereas it is abundant in semolina (Feillet et al., 2000; Ficco et al., 2014).

Feillet et al. (2000) found genetic controls POD more than the environment in common wheat; however, in durum wheat both environment and genetic control POD activity. Among all carotenoid degradation genes in durum wheat, there has been a lack of attention paid to the POD genes (Colasuonno et al., 2019). The genes responsible for POD activity are mainly located on chromosomes 1, 2, 3, 4, and 7 (Kobrehel and Feillet, 1975; Benito and de la Vega, 1979; Bosch et al., 1987; Liu et al., 1990; Wei et al., 2015). Among all POD isoforms that were found in durum wheat, isoform *Per-5* has the largest effect on pasta browning due to its specific location (endosperm) (Feillet et al., 2000; Fraignier et al., 2000; Colasuonno et al., 2019). However, in a study conducted by Asisns and Perez de la Vega (1985), *Per-4* isoform on chromosomes 4 and 7 has the largest effect on brown color in pasta products.

In the current study, there were no A-QTL found that controlled POD activity in POP1. The main reason for this result is that both parents (Joppa and D121118) had a low amount of POD. However, there were 90 DE-QTL associated with POD enzyme activity in this population. The DE-QTL with high LOD (range between 20 to 25) have a more positive additive by additive effect on POD enzyme activity. One pair of DE-QTL on chromosome 2A-1 could reduce the amount of POD by 3.5 (U/g). These minor

DE_QTL could explain the high phenotypic variances for this population. These results support the Li et al. (2011) study that demonstrated epistatic QTL have an important role in wheat flour color.

In POP2 at the Williston location, one major A-QTL was found on chromosome 3A. The QTL *D2.POD3AW.ndsu* with more than 53% of PVE on POD activity, could be caused by the *per-3* isoform, which is abundant in durum wheat embryos. This A-QTL came from Carpio, which had a high POD activity and low YPC and SBP. This increased POD in Carpio could be due to using whole kernel flour rather than semolina. Feillet et al. (2000) reported that bran contained 13 times more POD enzymes than other parts of the durum wheat kernel. The other two minor A-QTL were found on chromosomes 1A and 4A and support previous research (Kobrehel and Feillet, 1975; Benito and de la Vega, 1979; Bosch et al., 1987; Liu et al., 1990; Wei et al., 2015). The A-QTL *D2.POD1AW.ndsu*, with a small effect of 3.2% on phenotypic variation, came from Mountrail. The QTL D2.POD4AW.ndsu, with an effect of 3.4% of PVE, came from Carpio. Hu et al. (2020) reported the right flanking marker (Tdurum_contig5008_556) in *D2.POD1AW.ndsu* has an effect on flag leaf area in wheat. In another study by Lin (2017), the right flanking marker (IACX2890) in *D2.POD4AW.ndsu* was associated with pre-harvest sprouting resistance. In POP2, only 20 minor DE-QTL were detected to be associated with POD enzyme activity; this low number of DE-QTL could be because of the predominance of the major A-QTL.

The QTL that were detected in the current study could be used in the future by the durum wheat breeding programs. Based on our knowledge, there is no specific research on POD in durum wheat because of the complexity of this trait. More studies are needed on POD activity that also will include fine mapping of the major A-QTL found in this study.

Relationship Between Traits Related to Color in Pasta

When it comes to pasta, the bright yellow color is a characteristic highly valued by consumers. Yellowness in semolina is determined by the proportion of yellow to brown pigment contents in the flour mixture and enzyme activities such as PPO and POD that can modify the color of pasta (Borrelli et al., 2008). To find the relationship among these traits, it is better to look at both populations separately.

In POP1, Joppa, as a cultivar with a high yellow color pasta production (Cabas-Lühmann and Manthey, 2020), brings a higher amount of YPC to the population with two major A-QTL (more than 35% of PVE), six minor A-QTL (more than 26% of PVE), and a few numbers of DE-QTL. Joppa is a source for

all minor A-QTL for PPO enzyme activity and contributes more PPO phenotypic variation to the population (more than 26% of PVE). However, QTL for YPC presence in Joppa leads to masking the PPO enzyme activity QTL, reducing the total PPO activity in Joppa and increasing the yellow color. Experimental line D121118, another parent in POP1, does not have major or minor A-QTL for YPC, PPO, and POD enzyme activity and has a major A-QTL for SBP (more than 15% of PVE). The results on the experimental line D121118 indicate, in the absence of QTL for YPC and PPO enzyme activity, SBP is responsible for D121118`s dimmer color. In summary, Joppa has high YPC and low SBP, PPO, and POD enzyme activity and shows the effect of YPC QTL on other color-related traits. In POP2, Mountrail is high in SBP and low in YPC, PPO, and POD enzyme activity, these results indicate QTL for SBP could control the color of durum when QTL for YPC are not present.

In addition, in progenies (Entry line 78 [Table 2.3]) with major A-QTL for YPC and SBP and one minor A-QTL for PPO activity, the amount of YPC is high and the amount of SBP is low. These results show QTL responsible for YPC could mask the QTL that control SBP and PPO and decrease the brown color in durum wheat.

Although all of the color-related traits in Carpio are high (YPC, SBP, PPO, and POD), Carpio is considered a durum cultivar with a highly desirable yellow color (Elias et al., 2015). Carpio as one parent in POP2 with high yellow color (Cabas-Lühmann et al., 2021), has A-QTL for YPC with more than 38% of PVE, A-QTL for PPO with more than 35% of PVE, and A-QTL for POD with more than 53% of PVE. These results indicate that major A-QTL on chromosome 7A masks the effects of other QTL and increases the yellow color in Carpio. The major A-QTL on chromosome 3A (explaining more than 53% of PVE of POD activity) could not decrease the total YPC in Carpio. Also, major and minor A-QTL for PPO activity, with more than 35% of PVE on PPO activity, could not reduce the yellow color in Carpio.

Mountrail, as another parent of POP2, has a minor A-QTL for SBP (more than 8% of PVE), and has relatively high SBP (Table 2.4). Mountrail, as mentioned by Cabas-Lühmann et al. (2020), is scored as a bad cultivar for color-related traits. Mountrail is the only source for SBP in this population, and is considered a poor source for color-related traits. These results indicate in the absence of QTL for YPC, the QTL for SBP could lead to a reduction in the yellow color of durum wheat whole wheat flour and dimming the color of pasta.

In both populations, there are some DE-QTL responsible for more than one trait. Most of these DE-QTL control SBP and POD enzyme activity. These results indicate the same DE_QTL control SBP and POD enzyme activity and also explain why these traits together could increase the brown pigment in durum wheat and dim the color of pasta.

Our results show that YPC is more important than other color-related traits (SBP, PPO, and POD). In the presence of major and minor QTL for YPC, QTL of other traits could not affect the color. Our results support Ficco et al. (2014) and Cabas-Lühmann and Manthey (2020), which explain that YPC has an important effect on pasta color. Also, Carpio and Joppa could be suitable sources in the NDSU breeding program for high yellow color.

Conclusion

Overall, all parental lines (Joppa and D121118 in POP1 and Mountrail and Carpio in POP2) contributed desirable and undesirable alleles for color-related traits. Among all four parents, Joppa and Carpio could be valuable resources for improving color in durum wheat breeding programs. In the current study, based on our knowledge, it is the first time that a high-density SNP-based linkage map has been established and used to discover QTL for SBP in durum wheat.

This study found six stable major main effects A-QTL, 25 minor A-QTL and 370 minor DE-QTL associated with color-related traits in durum wheat. These results suggest major and minor A-QTL and minor DE-QTL should be considered for these traits in molecular wheat breeding programs.

The findings of this study, as well as the closely linked markers associated with the major A-QTL and minor DE-QTL found, could be extremely useful in determining the genetic nature of color-related traits in durum wheat. The detected QTL could be useful in marker-assisted breeding schemes to improve durum wheat color. The high-density maps that have been developed also provide a superior starting platform for fine mapping and, ultimately, map-based cloning of the major and stable loci that have been found in this study.

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CHAPTER 4. GENERAL CONCLUSION

Durum wheat is the world's second most important wheat, and the United States is one of the major producers. North Dakota is the top producing state, accounting for more than half of the durum wheat production in the United States. The majority of durum wheat is utilized in the manufacturing of pasta. The bright yellow color of pasta is an important trait among customers. The proportion of yellow and brown pigments in the flour and enzyme activity such as polyphenol oxidase (PPO) and peroxidase (POD) can determine the yellowness of semolina and change the color of pasta. This dissertation aimed to uncover new genomic regions associated with color-related traits and make these discoveries easier to select for in durum wheat breeding programs.

Yield, TWT, and all of the color-related traits revealed significantly different values between the parental lines. The results in chapter two identified two inbred lines in POP1 (Entry lines 32 and 78) with high YPC and low SBP and in POP 2 entry lines 1, 82, and 101 with high YPC, indicating RILs with combinations of desirable alleles from both parents at specified loci could be useful in breeding programs to improve pasta color. In this QTL-mapping study, 31 A-QTL (6 major A-QTL and 25 minor A-QTL) and 370 DE-QTL were identified, indicating that different genes in tetraploid wheat control color-related traits such as YPC, SBP, PPO, and POD. These A-QTL and DE-QTL can be used with MAS selection to develop durum wheat cultivars with the desirable amount of yellow color in end products. All parental lines (Joppa and D121118 in POP1 and Mountrail and Carpio in POP2) contributed desirable and undesirable alleles that affected color-related traits.

Our results show that YPC is more important than other color-related traits (SBP, PPO, and POD). In the presence of major and minor QTL for YPC, QTL of other traits did not affect the color. The results of this current study imply that the durum wheat cultivars Joppa and Carpio, developed by the NDSU durum wheat breeding program, are an excellent source of high yellow color since they have the major QTL for YPC and do not have undesirable QTL for SBP.

Additionally, this study reveals to produce a high yellow color in pasta products, it is necessary to breed out some unwanted QTL for SBP, PPO, and POD enzyme activity. A fine-mapping analysis using this genomic information could possibly lead to future map-based gene cloning of these desirable and

undesirable QTL and provide a more efficient resource for employing genomic techniques to select durum wheat with high yellow color.