7 Molecular Mapping and Breeding of Physiological Traits

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1 INTRODUCTION

1.1 Genetics and Plant Breeding

An understanding of genetics has been the key to improving our knowledge of many aspects of biology. Plant breeding has advanced greatly through an understanding of the principles of heredity, with Mendelian genetics forming the basis of plant breeding. This together with an understanding of biometrical genetics which applies to traits showing continuous variation and controlled by more than one gene, has allowed the manipulation of quantitative traits. Plant breeding aims to develop cultivars which fit specific environment and production practices and high yielding products whether for food, feed or processing into another product. Progress in any breeding program is based on the amount of genetic variability available and how effective is the selection and evaluation of the trait in question. Selecting for improved phenotype began early in the domestication of plants and has been the primary means ever since. Figure 1 shows the diversity of different Peruvian landraces of potato through selection for desirable traits. Phenotypic selection has limitations especially when interest is

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Fig. 1 Phenotypic variation caused by genetic diversity of potato tubers from Peruvian landraces. Courtesy of SCRI.

focused on more complex physiological traits. A more accurate way of selection would be at the genetic level where markers linked to the gene(s) or quantitative trait loci (QTLs) underlying the trait can be screened for. A prerequisite for genotypic selection is the establishment of associations between traits of interest and genetic markers.

1.2 Nature of Physiological Traits

Plant physiology is the study of the functions and processes occurring in plants, which include the various aspects of plant life such as yield, photosynthetic capacity, cold tolerance, water relations, mineral nutrition, growth, developmental stages, and response to environmental stimuli as well as their interactions *inter se*.

Understanding the genetic control of physiological traits and the linkage of these physiological characteristics to molecular markers on chromosomes, and ultimately the gene(s) underlying the trait is the future of plant breeding. Mapping and cloning of genes involved in

various physiological traits is a rather complicated process though due to the complexity of plant functions. In contrast, the accelerated pace of gene sequence discovery has surpassed our capability to understand the biological function of many genes. Sequencing technology has provided a wealth of genomic (total DNA) and expressed sequence (DNA that is transcribed and translated into protein) information, that has been accompanied by projects to locate, annotate, and assign biological function. Despite these efforts only a fraction of these annotated genes are associated with a phenotype that provides a predictive framework for understanding and manipulation. Complex traits such as pest and disease resistance mechanisms and quality traits such as flavor, texture, and appearance are poorly understood at the molecular/biochemical level. A renewed focus on linking genes to phenotypes is required to ensure our understanding of commercially acceptable traits.

2 MOLECULAR MARKERS FOR PHYSIOLOGICAL TRAITS

Molecular markers are DNA sequences (both known and unknown) that are located near genes and inherited characteristics of interest, allowing selective breeding and identification of progeny with desired characteristics. Molecular markers have been rapidly adopted by researchers globally as an effective and appropriate tool for basic and applied studies addressing physiological traits. Markers are most informative when integrated into the genetic linkage maps. Molecular markers, their history, type, and applications are described in detail in Chapter 2 in Volume 1.

These molecular markers are used as tools that identify DNA polymorphisms between DNA samples of different individuals. These polymorphisms can be of many different types from single nucleotide changes, large or small insertions and deletions or length variation in repeat sequences. All, however, provide information on that particular locus in the genome and importantly when that locus is known to be associated with a particular plant phenotype. An important way of linking marker loci to a particular plant phenotype is through the use of genetic linkage maps (Figure 2). These maps when coupled with field trails and glasshouse or laboratory experiments to measure traits of interest on the population of individuals used for map development can then be used to relate phenotypic data to marker data on linkage maps (Graham et al. 2006).

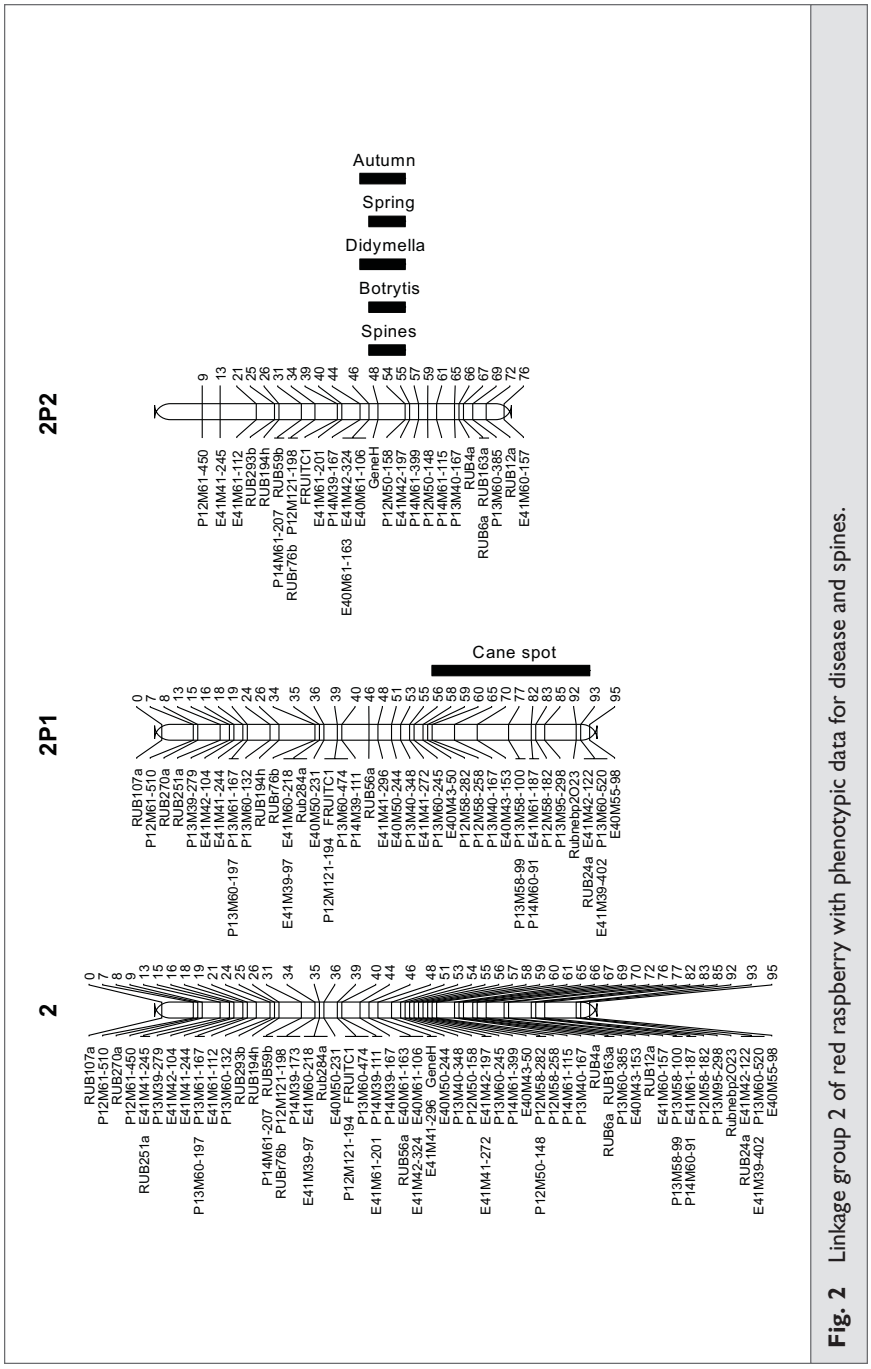

Fig. 2 Linkage group 2 of red raspberry with phenotypic data for disease and spines.

For map construction, individual marker loci are genetically characterized in a segregating population and the recombination rate of alleles at each pair of loci can be determined using classical linkage analysis. Loci can then be ordered into a linkage map and distance between loci can be expressed as recombination units given in centiMorgans (cM) where one cM is equal to 1% recombination. Once a sufficient number of markers have been mapped, the number of linkage groups should equal the haploid number of chromosomes. Several computer programs are available to quickly generate a map once markers have been applied to a segregating population. A detailed description of map construction is given in Chapter 4 in Volume I.

In the initial phase of map creation, genetically diverse parents are chosen which are known to segregate for the trait(s) of interest and depending on the biology of the crop an F_1, F_2, or backcross used for map construction. Once a map and segregating population have been developed attempts can be made to identify map locations of traits of interest. The speed and precision of crop enhancement can be improved by the development of genetic linkage maps which allow us to develop diagnostic markers for polygenic traits and in the future, aid the identification of the genes behind the traits (Figure 3).

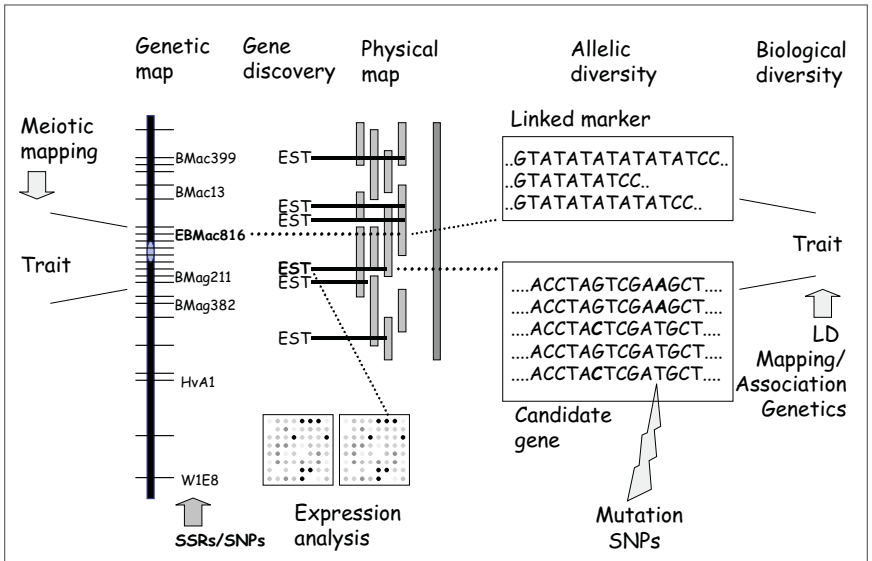

Fig. 3 Integration of different approaches to understand the genes controlling a given phenotype.

3 QTL MAPPING OF PHYSIOLOGICAL TRAITS

QTL mapping of physiological traits will provide crop breeders with a better understanding of the basis for the genetic correlation between economically important traits. This has potential to facilitate a more efficient incremental improvement of specific individual target traits.

Physiological traits may not be routinely screened for as the resulting phenotypes are time consuming or expensive to determine. Once experiments have been carried out however and DNA markers have been identified closely linked to the QTL involved in the expression of a physiological trait, they can be used efficiently in future marker-assisted breeding experiments. Identification at the same genomic location of QTLs related to physiological and morphological traits should be expected, given that changes in physiological pathways have an impact on the plant phenotype. Mapping single traits and QTLs are described in detail in Chapters 5 and 6, respectively in Volume 1. Once markers are generated, they can be used swiftly on various populations using marker-assisted selection (MAS). QTL mapping has become a standard procedure in dissecting the genetic controls of a variety of traits (see Table 1, for examples). The next step is the identification of the genes, alleles and physiological processes that are biologically important. In this chapter, some important physiological traits which have been studied at the molecular level are discussed to highlight the potential for marker-assisted breeding even for complex traits.

For crop plants often the most important trait from a plant breeder, grower and end user perspective is yield. This 'single' trait is in fact the end result of many physiological processes which may appear directly related or remote, but all interacting at some level to give the final biomass of the crop. An understanding of physiological processes and how they interact to produce the end product is a goal towards which QTL mapping can play a part both in basic research into a process as well as in applied breeding programs leading to an enhanced crop variety. From what is regarded as the most fundamental of plant processes such as photosynthesis, through growth and development to what may appear to be of more secondary functions such as response to stress, all have important implications for crop yield.

Through a number of different physiological processes, examples of how QTL mapping can and is being applied to increase understanding of plant physiology and will subsequently impact on plant breeding will be demonstrated. This will cover aspects of photosynthesis, nutrient absorption and assimilation, nitrogen fixation, senescence, flowering, and stress.

Table 1 Selected examples of some of the important traits for which genetic marker techniques have been used to map loci of physiological traits.

Crop	*Trait*	*Reference*
Rice	Ion transport	Koyama et al. 2001
Barley	Photoperiod response	Stracke 1998
	Photoperiod, vernalization	Karsai et al. 1997
Wheat	Flowering time	Tóth et al. 2003
	Flowering time	Sarma et al. 1998
	Vernalization	Sutka et al. 1999
	Flowering, physio-morphological traits	Börner et al. 2002
	Nitrogen absorption	Kato et al. 1999
	Metabolism, physiological traits	Gao et al. 2004
Pearl millet	Flowering, ear emergence time	Yadav et al. 2003
	Flowering, ear emergence time	Kato et al. 1999
Ryegrass	Heading date	Armstead et al. 2004
Soybean	Flowering, oil, protein	Zhang et al. 2004
Brassica oleracia	Flowering	Sebastian et al. 2002
Brassica rapa, Brassica napus	Flowering time, vernalization, winter survival, freezing tolerance	Kole et al. 2001, 2002
Sunflower	Flowering	Bert et al. 2003
Lotus, pea	Nitrogen fixation	Stracke et al. 1998
Solanaceae	Flowering	Frary et al. 2003
Potato	Carbohydrate metabolism	Chen et al. 2001
Sugar beet	Sucrose content	Schneider et al. 2002
Arabidopsis	Flowering	Loudet et al. 2002
	Carbohydrate and nitrogen metabolism	Rauh et al. 2002
Sugi	Flowering, juvenile growth	Yoshimaru et al. 1998

3.1 Photosynthesis

Photosynthesis is the process by which plants, algae, and some bacteria use the energy from sunlight to produce sugar, which cellular respiration converts into ATP, the 'fuel' used by all living things. So, for example in rice, photosynthesis is the primary source of dry matter production and grain yield. The contribution of leaf photosynthesis to biomass has been estimated at 30%.

For uniformity throughout the book, have put quotations in " " and emphasized words in ' ' in all the chapters

The conversion of unusable sunlight into usable chemical energy is associated with the actions of the green pigment chlorophyll. Most of the time, the photosynthetic process uses water, releasing oxygen essential

for life. The raw materials of photosynthesis, water and carbon dioxide, enter the cells of the leaf, and the products of photosynthesis, sugar and oxygen, leave the leaf. The process is complex with many steps and many factors contributing to efficiency. Leaf photosynthesis cannot be measured in the field; hence the trait does not lend itself to large scale screening of segregants in a breeding program. If the process can be broken down, however, into the various processes and factors of importance, attempts can then be made to map these factors. Thus, a number of phenotypes and processes that impact on the photosynthetic potential of a plant can be measured. Factors such as chlorophyll content, gas diffusion, water availability, leaf size, root properties, and temperature, to name a few, will all have a role in determining the plants achievable photosynthetic potential. Research aimed at mapping QTLs relevant to photosynthetic potential has been initiated.

Recently a group working in rice (Teng et al. 2004) measured photosynthetic rate, chlorophyll content, stomatal resistance, and transpiration rate from the parents and a doubled haploid (DH) population containing 127 lines. A map was established from this population using restriction fragment length polymorphism (RFLP) and simple sequence repeat (SSR) markers and from this, two putative QTLs for net photosynthetic rate, three QTLs for chlorophyll content, one for stomatal resistance and two for transpiration rate were identified.

The interrelationship between photosynthesis and other physiological traits has been highlighted in works on both low temperature and water stress (discussed in more detail below). Low growth temperature parameters are considered to be important on the photosynthetic apparatus. In particular the combination of high light intensity and low temperature can cause photosynthetic inhibition of photosynthesis. This could be due to a number of factors including the ability to develop a functional photosynthetic apparatus at low temperature, as well as the susceptibility of enzymes in the C_4-cycle. In a study of maize grown under chilling stress, photosynthetic performance as well as shoot biomass was affected under low temperature and QTLs were mapped for a number of parameters (Jompuk et al. 2005). The photosynthetic traits net CO_2 uptake, stomatal conductance, and abscisic acid (known to have a role in regulating stomatal opening) have also been measured in relation to water stress. Here the authors looked at normally irrigated and water-derived young maize plants and found that QTL distribution was strongly influenced by water stress (Pelleschi et al. 2006). These examples begin to illustrate the relationships between traits and their final manifestation as yield potential.

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3.2 Nutrient Absorption and Assimilation

Green plants are by far the earth's most important autotrophic organisms which can subsist in an inorganic environment by manufacturing their own organic compounds from raw materials small enough and soluble enough to pass through cell membranes. As seen in the last section, the raw materials most needed for photosynthesis are carbon dioxide and water which supply carbon, oxygen, and hydrogen, the predominant components in organic molecules. Carbon dioxide is obtained from the air through the leaves and water from the soil through the roots. There follows the synthesis of carbohydrate making up the bulk of dry weight of the plant. Other elements enter into the composition of the plant. Nitrogen is present in amino acids and two important amino acids also contain sulfur. ATP contains phosphorous and chlorophyll contains magnesium to name a few other elements. These other elements must therefore come from the soil, hence the development of fertilizer regimes in agriculture. Modern fertilizers are often designated by their ratios of N-P-K, these three elements are most rapidly removed from the soil and need to be replenished if crops are to continue to flourish. Functions of leaves and roots essential to nutrient absorption must therefore impact greatly on plant biomass. Thus for roots the capacity for water uptake is fundamental for crop productivity. The capacity of the root for uptake depends on the degree to which the root extends its absorption area, which is determined by complex root morphology. The lateral roots which occupy more than 90% of the total length of the root system play a major role in water uptake. To date studies have been carried out to identify QTLs related to root morphological features such as root mass and depth, root axis length and lateral branching (Yadav et al. 1997; Shen et al. 2001; Horii et al. 2006). QTLs for other factors, such as the effects on the plant of boron toxicity at high concentrations, have been identified (Jefferies et al. 2000). In studies on grain yield, differences in the concentration of boron have led to a 17% difference in grain yield. Other areas such as the ability to increase uptake of applied nitrogen fertilizer, whereby reducing losses into the environment have been studied (An et al. 2006). Understanding and optimization of plant nutrient uptake, therefore, have great potential for ultimate yield improvement not to mention other aspects such as tolerance to toxic compounds in the soil.

3.3 Nitrogen Fixation

Although the earth's atmosphere contains 78% nitrogen, free gaseous nitrogen cannot be utilized by animals or by higher plants. They depend

instead on nitrogen that is present in the soil. To enter living systems, nitrogen must be 'fixed' (combined with oxygen or hydrogen) into compounds that plants can utilize, such as nitrates or ammonia. A certain amount of atmospheric nitrogen is fixed by lightning and by some cyanobacteria blue-green algae. But the great bulk of nitrogen fixation is performed by soil bacteria of two kinds: those that live free in the soil and those that live enclosed in nodules in the roots of certain leguminous plants (e.g., alfalfa, peas, beans, clover, soybeans, and peanuts). The symbiotic interaction between legumes and rhizobial bacteria accounts for a significant portion of biological nitrogen fixation worldwide. Nitrogen fixation is the process by which atmospheric nitrogen gas is converted into ammonia. The ammonia is subsequently available for many important biological molecules such as amino acids, proteins, vitamins, and nucleic acids. Legumes have the ability to use certain kinds of bacteria (genus *Rhizobium*) as a means of getting nitrogen through a process of nitrogen fixation. The site of fixation is the nodule, a unique plant organ located on the root, which functions to generate the aerobic environment essential for bacterial survival and nitrogenase activity. Nodule formation involves plant/bacterial signaling, with the bacterially generated signaling molecule, Nod factor, playing a critical role. The bacteria and the plant have a symbiotic relationship; the plant provides the bacteria with food, and the bacteria fixes nitrogen for the plant releasing much of the fixed nitrogen into the plants cytoplasm primarily in the form of amino acids. The bacteria in nodules generally produce a surplus of fixed nitrogen some of which is excreted from the roots into the soil. The whole nitrogen fixation process is very complicated because it involves both the bacterium and the plant. The process begins by host recognition followed by in most cases root hair infection. This leads to nodule development and to the reduction of nitrogen to ammonia catalyzed by an enzyme complex known as nitrogense. The plant genes determining nodulation and nitrogen fixation are not well known. Mapping out the genetic blueprints of the specific proteins and enzymes involved in the process allows us to understand how nitrogen fixation is regulated. Identification of the symbiotic genes controlling nodule formation and the efficiency of nitrogen fixation are one of the main goals in legume breeding. It involves localization of symbiotic genes on chromosomes with the use of genetic, biochemical, and ultrastructural analyses of symbiotic mutants.

Some nodulin genes and plant proteins with functions in oxygen transport, cell wall architecture, sugar, and N metabolism have been identified (Verma et al. 1992). A number of mapping studies have been carried out in this area. In common bean, QTLs affecting nodule number

have been identified (Nodari et al. 1993). In soybean, QTLs controlling nodulation and shoot mass have been determined (Nicolas et al. 2005). *Sym* genes are essential for nodulation and/or symbiotic nitrogen fixation in legumes. Two novel *Sym* genes were isolated in *L. japonicus* by combining genetic and physical mapping with genome sequencing (Stracke et al. 2004). Approximately 40 *sym* mutants have been identified to date in various legume species, including pea, soybean, *M. truncatula*, and *L. japonicus.* Mapping QTLs and identification of regulatory genes are greatly increasing knowledge of the nitrogen fixation process.

3.4 Senescence

The biological importance and potential for improvement of crop characteristics, particularly plant productivity and post-harvest storage, have prompted extensive physiological, molecular, and genetic analyses of leaf senescence. Senescence is considered the final stage in leaf development. It is an active, ordered process that involves mobilization of nutrients from the senescing leaves to other parts of the plant, leading to the eventual death of the leaf. Plant hormones such as ethylene and cytokinins play vital roles in the regulation of senescence. Attention has focused on cytokinins that are key components of plant senescence. These compounds have been implicated in several aspects of plant development and are thought to be synthesized mainly in the roots and transported to the shoots via the xylem. Three main approaches have been used to study the effect of cytokinins in plant senescence, namely exogenous application of cytokinin solutions, measurement of endogenous cytokinins during senescence, and transgene-encoded cytokinin biosynthesis. Enzymes with varied functions have also been found to be involved in senescence. Senescence is a genetically programmed sequence of biochemical and physiological changes, a type of programmed cell death characterized by loss of chlorophyll, lipids, total protein, and RNA. The initiation of senescence is subject to regulation by various environmental and autonomous (internal) factors. Plants have evolved mechanisms by which leaf senescence can be induced by many stresses to reallocate nutrients to reproductive organs and to eliminate water consumption by older, less productive leaves. This regulation of leaf senescence has an obvious adaptive value as it allows the plant to complete its life cycle even under stressful condition. Senescence is a correlative event and is the cumulative effect of the formation of flowers, fruits, seeds, apices, and roots. Senescence can be induced by environmental stress, such as low light intensity, nutrient deficiency, pathogen attack, drought, waterlogging, and detachment

from the plant. Endogenous factors, including leaf age and reproductive development, also trigger senescence. The identification of genetic mutants that control leaf senescence in *Arabidopsis thaliana* opened up new possibilities for genetically analyzing leaf senescence in a model system. ORE9, an F-box protein that regulates leaf senescence in Arabidopsis was identified by a map-based cloning approach. Genetic mapping, using cleaved amplified polymorphic sequence (CAPS) markers, placed *ORE9* locus on chromosome 2 (Woo et al. 2001).

Manipulation of leaf senescence may play an important role in crop production. For example, late leaf senescence has been shown to play an important role in increased photosynthetic activity in plants (Mae 1997). In rice, 60-90% of the total carbon found in rice panicles is produced by photosynthesis after heading. Genetic analysis of the trait has been hindered by the difficulty in measuring this trait. In a recent study, QTLs for senescence were identified by measuring leaf chlorophyll content, reduction in chlorophyll content and the number of late-discoloring leaves per panicle (Farouk et al. 2005). Not listed in references

3.5 Flowering

Plant development is seasonal with flowering and other developmental processes occurring at particular times of the year. The obvious explanation for this timing is that these stages happen in response to environmental cues such as temperature or light intensity. These seasonal changes are in fact dependent on certain environmental characteristics acting as inductive signals to set in motion complex developmental processes. Photoperiodism, response to the length of day and vernalization, response through exposure to a period of cold, are two major mechanisms underlying such seasonal responses. Response to day-length and vernalization are two of the most important mechanisms controlling flowering, and therefore yield, in many of our crop plants. Flowering time is determined by genes that control the vernalization response and also by genes that control the photoperiodic response or affect flowering time independently of photoperiod and vernalization. Individual genes which have qualitative effects on these responses have been identified using molecular markers. The complex interactions among genes of these three classes often result in continuous variation in flowering time that is usually analyzed by quantitative trait loci (QTL) techniques (Laurie et al. 1995). Mapping of flowering time genes in relation to molecular markers is clearly a workable method and a consequence of colinearity is that markers mapped in other crosses or species become available for use.

The time of flowering–in some crops referred to as heading date–is a key target trait in breeding programs for many crop species. Flower development consists of several phases.

(1) The first step is the transition from vegetative to reproductive development, regulated by floral induction.

(2) Later steps include the initiation of individual flowers, the determination of organ identity, and organ-specific differentiation

In most plants flowering is triggered by either day-length, by exposure to a period of low temperature, or by both. The plant has parameters for the optimal, minimum and maximum temperatures for floral induction. Plants can be placed into three general groups based on their response to photoperiod (their requirement of light for initiation of flowering).

(1) *Short-day* plants such as corn require long night lengths to flower.

(2) *Long-day* plants such as lettuce require short night lengths to flower.

(3) *Day-neutral* plants such as tomatoes do not depend on photoperiod to flower, but high or low temperatures can retard flowering.

When the required photothermal units (the optimum amount of light and temperature) for floral induction are accumulated, the plant moves into the floral initiation phase. Floral initiation is the stage when the plant has decided to flower and is making the biochemical changes necessary to create inflorescences, flowers and fruits (Figure 4). The length of the floral initiation period depends largely on temperature in many plants. Flowering time can be assessed in the same way as many other phenotypic variables by genetic mapping of segregating populations developed from parents with different attributes. In cases where genes of large effects are segregating, flowering time in a population may be resolvable into discreet Mendelian classes. In other cases where the trait behaves in a quantitative manner the underlying genes (QTL) must be located by statistical methods. Linked markers can be used for selection of specific flowering time alleles, but because their efficiency depends on the tightness of linkage they will be most efficient for genes that are accurately mapped. There are well established common principles that link crop model species and in several cases orthologous genes have been shown to regulate equivalent developmental process.

The most striking recent advances in our understanding of the genetic control of the timing of flowering have come from work on Arabidopsis. A large number of genes that control the timing of the

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Fig. 4 Flowering in blackberry (*Rubus* spp) Courtesy SCRI.

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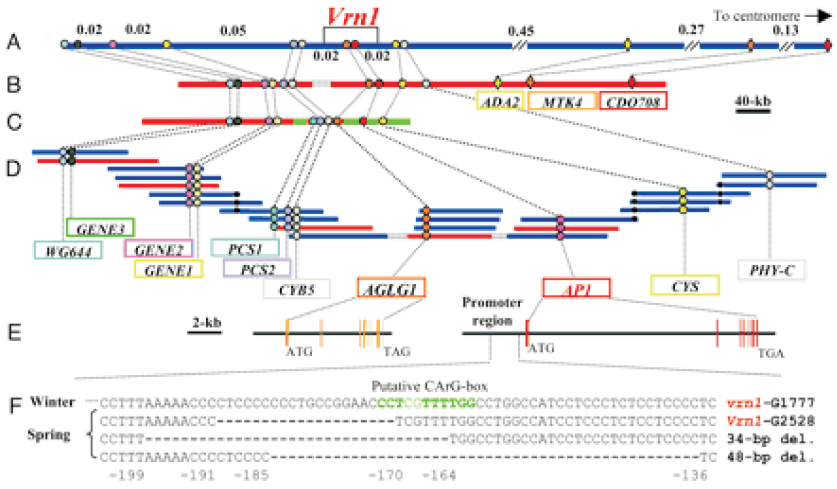

Fig. 5 Genetic and physical map of the *VRN1* region on chromosome $5A^m$ of *T. monococcum* (Yan et al. 2004).

transition to flowering have been identified in Arabidopsis by mutant analysis Genetic analysis of flowering time in pea, cereals, and Arabidopsis supports the hypothesis that the transition to flowering is under multifactorial control. Multiple genes that control flowering time have been identified in different species.

The understanding of the genetic control of flowering time is much more advanced in Arabidopsis than in crop species. Due to their close phylogenetic relationships, information about Arabidopsis flowering time can be readily exploited in *Brassica* species. In these models flowering is regulated by photoperiod, vernalization, and gibberellin pathways. Identification of the genes involved in flowering time has made it possible to determine the genetic control pathways for the response to photoperiod and vernalization in Arabidopsis (Levy and Dean 1998, Samach and Coupland 2000). In addition, homologs of Arabidopsis genes for flowering time also function in *Brassica napus* and *B. rapa* (Robert et al. 1998; Kole et al. 2001). Identification of the genes involved in flowering time has made it possible to determine the genetic control pathways for the response to photoperiod and vernalization in Arabidopsis Numerous studies have concentrated on mapping genome regions controlling flowering time and vernalization requirement (e.g., Ferreira et al. 1995, Kole et al. 2001, 2002)

In cereals, understanding the genes that control flowering time is best understood in rice (Yano 2001; Yano et al. 2001; Izawa et al. 2003; Hayama and Coupland 2003). Several flowering time genes have been isolated from rice, principally by positional cloning methods. Control of flowering in response to day-length has been analyzed extensively, and interestingly the genes cloned to date relate directly to known genes in Arabidopsis. Rice is very different from Arabidopsis in flowering behavior, being a tropical species that shows promotion of flowering in response to short-days and lacks a vernalization response. Rice is a short-day (SD) plant; its heading is promoted by short photoperiods. The response of the plant to length of day (referred to as photoperiod sensitivity) and its basic vegetative growth determine the heading date of rice. Heading date is a critical trait for adaptation to different cultivation areas and cropping seasons. Like many other important traits in plant breeding, heading date is a complex trait that shows continuous phenotypic variation among progeny and is controlled by multiple genes known as quantitative trait loci (QTLs). QTLs typically are difficult to identify because of the lack of discrete phenotypic segregation and because the phenotypic effects of each gene associated with a complex trait are relatively small. A major quantitative trait loci (QTL) controlling response to photoperiod, *Hd1*, was identified by means of a map-based

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cloning strategy (Yano et al. 2000). Many genes for heading in rice have been genetically identified (Yokoo et al. 1980; Yamagata et al. 1986; Poonyarit et al. 1989; Yokoo and Okuno 1993; Tsai 1995; Kinoshita 1998; Yamamoto 1998, 2000). The major genes or quantitative trait loci (QTLs) for heading date have been mapped by using DNA markers (Mackill et al. 1993; Xiao et al. 1996; Yano et al. 1997; Lin et al. 1998; Tamura et al. 1998).

Photoperiod sensitivity genes which control growth phase of rice plant from the vegetative stage to the reproductive stage are very important in breeding programs of rice. *Se-1* gene, one of the major genes for the photoperiod sensitivity of rice has been located on chromosome 6. In barley, two major loci regulating photoperiod response have been identified by genetic analyses (Laurie et al. 1995). Major loci controlling photoperiod response have also been mapped to the short arms of the group 2 chromosomes of wheat and comparative mapping using common RFLP markers shows that the wheat and barley genes fall into equivalent intervals.

Not all plants have a vernalization requirement, and the degree of vernalization required can vary within a species. In many plants, flowering can be accelerated or induced by exposure to a long period of near-freezing temperatures. This is a commonly employed reproductive strategy that allows for flowering and seed production in the environmentally favorably period following natural winter. Vernalization, has been studied for decades at the physiological level but only recently at the molecular level. The isolation of *FLOWERING LOCUS C*, in Arabidopsis has now provided an insight into the molecular mechanism involved, including the role of DNA methylation. The MADS-box protein encoded by *FLOWERING LOCUS C* (*FLC*) is a repressor of flowering. Vernalization, which promotes flowering in the late-flowering ecotypes and many late-flowering mutants, decreases the level of FLC transcript and protein in the plant. Many plants are responsive to vernalization, and use it as a cue to indicate that winter has passed ensuring flowering occurs in the favorable conditions of spring. Many late flowering Arabidopsis accessions and mutants exhibit a strong vernalization response. The roles of abscisic acid (ABA), gibberellins (GAs) and phytochrome B in the vernalization response have been investigated by combining mutations causing defects in their biosynthesis and response with *fca-1* (Chandler et al. 2000).

Winter wheat require several weeks at low temperature to flower. This process is controlled mainly by the *VRN1* gene (Yan et al. 2003). Genes controlling vernalization requirement in hexaploid wheat and other temperate cereals have been extensively characterized. Genes for

vernalization response have been located in chromosomes of homeologous group 5 of wheat (Snape et al. 2001). Figure 5 shows the genetic and physical map of *VRN1* region of *T. monococcum.* Not listed in references

Three major loci controlling vernalization response have been identified in barley by genetic analysis. *Vrn-H1* maps to the middle of the long arm of barley chromosome 5H and comparative mapping shows that major vernalization response genes map to equivalent positions in wheat (*Vrn-A1*, *-B1,* and *-D1*) and rye (*Vrn-R1*; Laurie et al 1997; Börner et al. 1998). Not listed in references

Photoperiod and temperature are important for initiating pathways of development. As we have seen above in photosynthesis these parameters impact on photosynthetic potential.

3.6 Stress Tolerance

Plants are faced with a large number of stresses and have developed many physiological pathways to cope with them. Current breeding is hampered by a lack of understanding of the genetic mechanisms responsible. A large number of genes, transcripts, and proteins implicated in stress tolerance have been identified though their function remains unclear.

3.6.1 Salt Tolerance

It is estimated that saline soils cover between 400 and 950 million hectares of the Earth's surface. Accumulation of salt in the soil causes deleterious effects leading to a reduction in production from rice and other crops. Salt tolerance of a crop is the final manifestation of several components such as Na^+ and K^+ uptake and exclusion, ion balance, and ion compartmentalization. For example salt tolerance in rice is an important objective of rice breeding for coastal areas. Yeo et al. (1990) dissected the complex physiological trait of salt tolerance by measuring physiological components such as shoot sodium content, plant survival, and plant vigor. With the development of molecular marker technology the potential for genetic dissection of the mechanisms of salt tolerance are possible. QTL analysis of salt tolerance in rice has been carried out by several groups. These have identified QTLs for plant survival, Na^+ and K^+ uptake, Na^+ and K^+ concentration and Na+:K+ ratio in shoots (Zhang et al. 1995; Lin et al. 1998; Gong et al. 1999; Prasad et al. 2000; Koyama et al. 2001). Not listed in references

A QTL mapping approach concerning both shoots and roots has been carried out in rice and a number of QTLs identified. QTLs for Na^+

and K^+ concentration in shoots explaining over 40% of the phenotypic variation were detected (Lin et al. 2004). The QTLs however detected between the roots and shoots did not share the same map location suggesting different genes may be controlling the transport of Na+ and K+ between roots and shoots. A rice QTL, *SKC1* which maintains K+ homeostasis in a salt tolerant variety under salt stress has been mapped, and map based cloning carried out to isolate the gene which was shown to encode a sodium transporter (Ren et al. 2005).

3.6.2 Drought Tolerance

The development of drought tolerance in plants has been slow due to a lack of understanding of the physiological parameters that are diagnostic of genetic potential for improved productivity under water stress. Tolerance to drought involves a complex of mechanisms working in combination to avoid or to tolerate water stress (Tuberosa et al. 2002). Water deficit occurs when water potentials in the rhizosphere are sufficiently negative to reduce water availability to levels that are sub-optimal for plant growth and development. Plants under drought stress demonstrate a number of physiological responses. Osmotic adjustment (OA) has been found to occur in plants with resistance to water stress. Relative water content is a useful measurement of plant water status as affected by leaf water potential and OA. Other measures include water-use efficiency measured indirectly as carbon isotope ratio, abscisic acid levels, photosynthesis measurements (stomatal conductance and net CO_2 uptake) and ash content. QTLs have been identified for a range of water stress factors including morphological traits such as plant and leaf size, root properties and yield components to more specific evaluations such as leaf relative water content, leaf osmotic potential, osmotic potential at full turgor, water soluble carbohydrate concentration, osmotic adjustment, and carbon isotope discrimination as well as a number of crop productivity measures (Diab et al. 2004; Saranga et al. 2004). Not listed in references Candidate genes and ESTs have been identified underlying QTLs for drought tolerance (Diab et al. 2004).

3.6.3 Cold Tolerance

Exposure to low temperature early in development is essential for regulation of key processes as described above in 'Components of Flowering'. However this exposure can have a negative impact on the plant causing, for example poor photosynthetic performance. In maize, cold-induced decrease in photosynthesis has been associated to a number of changes such as damage to the PSII reaction centers (Haldimann et al. 1995), alteration of leaf pigment composition

(Haldimann et al. 1995) and lower enzyme activity in the carbon cycle (Kingston-Smith et al. 1997). The relationship between vernalization requirement and cold tolerance is unclear. Little is known about the genetic basis of cold tolerance and a better understanding would allow the identification of the complexity of different regulatory traits. Studies on different plant genotypes however have suggested there is considerable genetic variation for cold tolerance (Haldimann 1998). In a cross between a cold tolerant and a cold sensitive maize line, a number of significant differences in traits such as better tolerance to chronic photoinhibition, greater operating quantum efficiency and trapping efficiency of PSII, higher proportion of open reaction centers, higher carbon exchange rate and greater shoot dry mass were observed. QTLs for most of the measured traits were identified (Fracheboud et al. 2004).

4 CONCLUSION

Understanding physiological pathways and the many interactions between them has been one of the biggest challenges in plant improvement. An observed plant phenotype is the result of the expression of many diverse plant physiological and biochemical pathways. Given that most physiological tests cannot be applied routinely in a breeding program, because their protocols are generally too complex or time consuming, the genetic dissection of target traits and physiological parameters is of primary importance for plant breeding. In recent years, a number of practical examples have demonstrated the power of high-density genetic maps and candidate gene studies for the identification of genetic markers closely linked to important physiological traits. A recent review by Price (2006) has highlighted the accuracy of QTL mapping and where in fact, map-based cloning using the original data rather than fine-mapping is a realistic option for gene identification.

In Table 1—under barley—reference is Stracke 1998, however in references—Stracke, Borner 1998

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