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Genetic Architecture of Species level differences in *Begonia* Section Gireoudia

By
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DECLARATION

I declare that the work described in this thesis has been carried out by me unless otherwise acknowledged. It is entirely of my own composition and has not, in whole or in part, been submitted for any other degree.

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ABSTRACT

Begonia is one of the ten largest plant genera and is found throughout the tropics. I have used *Begonia* section Gireoudia to study the genetics underlying vegetative diversity in tropical herbaceous plants. Section Gireoudia is a large Central American group. The section is remarkably diverse in morphology and habitat preference. It ranges from wet rainforests to seasonally dry forests. I have investigated variation in morphological, anatomical and ecophysiological differences for 21 species in *Begonia* section Gireoudia. Based on the observed variation, species in *Begonia* section Gireoudia form a complex and unique group that stands out from currently analysed taxa in the global scale of variation on the basis of leaf function and resource use strategy traits as well as their peculiar leaf anatomy. Traits directly related to leaf function such as photosynthesis and stomatal conductance has very low values which overlap with those of CAM and aquatic plants. Values for traits indicative of resource use such as leaf mass area (LMA) and leaf dry matter content (LDMC) are also very low in *Begonia* when compared with the values observed globally. The trait- trait correlations across the species in section Gireoudia were also investigated and revealed patterns in micromorphology and ecophysiology.

Some of the traits measured are correlated with each other in apparently straightforward, well charaterised biological relationships e.g., the variation among *Begonia* species in stomatal conductance and net assimilation rate are positively correlated. On the other hand, the linkage of high A_{mass} with high N_{mass} which is in large part the result of a direct causal relationship, has been observed at the global scale but this relationship is not significant in *Begonia* section Gireoudia.

I examined *B. plebeja* and *B. conchifolia*, two very closely related though ecologically divergent species from Meso-America, in more detail. I detected significant differences between the species for a number of phenotypic variables which may be related to their habitat preferences. This suggested that environmental conditions have driven divergent evolution of phenotypic traits for these two species.

Using a mapping population generated from hybrids between these two species I was able to examine the genetic basis of these differences. This revealed that although some traits (such as anthocyanin accumulation) appear to be under simple genetic control, most of the variation between species has complex genetic inheritance patterns. I used QTL analysis to identify significant QTLs for 20 physiological, anatomical and morphological traits which varied between these two species. Leaf shape traits appear to be largely influenced by a few loci of large effect, making these good potential targets for further analysis. The study also identified clusters of coincident QTLs for different correlated traits identifying pleiotropic genes or suites of linked loci.

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Abbreviations and Symbols

A_{max}	**Maximal rate of net photosynthesis at ambient CO_2**
gs	**Stomatal conductance to water vapour**
Ci	**Internal CO_2**
Trmmol	**Transpiration**
SLA	**Specific Leaf Area**
LMA	**Leaf Mass Area**
Tleaf	**Leaf Temperature**
A	**Area of the Leaf**
P	**Perimeter**
C	**Circularity**
N. I	**Number of indents**
I w m	**Indent width (mean)**
I d m	**Indent depth (mean)**
FW	**Fresh weight**
DW	**Dry weight**
LDMC	**Leaf Dry Matter Content**
Spad	**Chlorophyll content (as assess by SPAD meter)**
% N	**percentage Nitrogen**
% C	**percentage Carbon**
Wl	**Width of lamina**
Wv	**Width at vein**
Wde	**Width of adaxial epidermis**

Wdh	**Width of adaxial hypodermis**
Wdc	**Width of adaxial cell**
Ndhc	**Number of adaxial hypoderm cells**
Tdhc	**Type of adaxial hypoderm cells**
Wbe	**Width of abaxial hypodermis cells**
Wbh	**Width of abaxial hypoderm**
Wbc	**Width of abaxial hypodermis cells**
Wm	**Width of mesophyll**
Ms	**Mesophyll structured**
Dpm	**Depth of pallisade mesophyll**
dsm	**Depth of spongy mesophyll**
aip	**Expression of anthocyanins in parenchyma**
NscF	**Number of stomatal clusters per mm^2 of leaf**
AV	**Average distance between stomatal clusters**
SSCW	**Substomatal cluster width**
SSCL	**Substomatal cluster length**
Nsc	**Number of stomata in each cluster**
Ast	**Arrangement of stomata**
bTt	**Abaxial type of trichomes**
blt1	**Abaxial length of trichomes type 1**
bNt1	**Abaxial number of trichomes type 1**
blt2	**Abaxial length of trichome type 2**
bNt2	**Abaxial number of trichome type 2**
dTt	**Adaxial type of trichome**
dlt1	**Adaxial length of trichome type 1**
dNt1	**Adaxial number of trichome type 1**
dlt2	**Adaxial length of trichome type 2**

dNt2	**Adaxial number of trichomes type 2**
wp	**Width of petiole**
Ncvb	**Number of central vascular bundles**
Nivb	**Number of inner vascular bundles**
Novb	**Number of outer vascular bundles**
wivb	**Width of inner vascular bundles in cross section**
Lovb	**Length of outer vascular bundle in cross section**
wovb	**Width of outer vascular bundles in cross section**

CHAPTER 1.Introduction

1.1 Speciation

Early speciation studies regarded ecology as an important contributor to the speciation process. Darwin's original description of the process of natural selection (1859) supported the idea that speciation occurs through adaptation to different environments, as in the case of Darwin's finches.

During the first half of the twentieth century many ecologists and evolutionary biologists highlighted the role of ecology in speciation. Poulton's (1908) main focus was primarily ecological divergence. He discussed the relative importance of pre-mating barriers versus post-mating barriers. He considered premating barriers important to the process of speciation. Important contributions in the field of speciation were also made by Dobzhansky (1937) and Mayr (1942). Dobzhansky (1937) included ecological isolation in his list of isolating barriers between species and stressed the role of ecology and natural selection in post zygotic isolation. He stated that "the genotype of a species is an integrated system adapted to the ecological niche in which the species lives". Mayr (1947) also concluded in his classic paper "Ecological factors in speciation" that geographic isolation leads to the formation of segregated populations that experience different ecological conditions, leading to evolutionary divergence.

Edgar Anderson (1948) highlighted the contribution of habitat disturbance in the breakdown of species reproductive barriers and the generation of hybrids. The role of ecology in speciation was also not underrated by G. Ledyard Stebbins (1950) who wrote, "By far the most common type of isolation in both the plant and the animal kingdom is that resulting

from the existence of related types in different geographical regions which differ in the prevailing climatic and edaphic conditions". The focus of the great evolutionary researcher Verne Grant's, experimental work was ecological speciation caused by pollinator-mediated isolation in the phlox family (Polemoniaceae) (1949, 1994).

Nonecological theories involving chromosome rearrangements, genetic drift, and various types of founder effects drew the attention of many ecologists and evolutions in the later half of the Twentieth Century (Coyne and Orr, 1990). It was during this era that ecological speciation was greatly neglected. However in the past few years the focus has returned to adaptation. Ecologists and evolutionary biologists are using new tools and next generation sequencing (NGS) techniques to identify genetic loci responsible for adaptive evolution in non-model organisms (Stapley et al., 2010).

Ecologists and evolutionary biologists have been conducted to assess the contribution of habitat, pollinator and temporal divergence in promoting speciation (Arnold, 1997; Schluter, 2000). Among the three kinds of divergences facilitating speciation, the focus of most research to date has been pollinator divergence in plants and little attention has been paid to study the role of habitat divergence (Coyne and Orr, 1990; Wu et al., 2007).

1.2 Habitat divergence

Genetic changes in a plant population which makes them fitter in their habitats will usually make them less fit in other habitats. Restriction to a particular habitat can limit mate choice to other populations which thrive there and offspring will also be constrained in the habitats they will thrive in. A classical study of this process involves plants that are adapted to heavy metals on serpentine soils (Macnair and Christie, 1983).

Habitat divergence is considered important in speciation (Feder et al., 2012). In allopatry, selection and genetic drift leads to the divergence of geographically isolated populations (Mayr, 1963; Rieseberg and Welch, 2003). Such divergence is also reported to lead to reduced competition between isolated populations in case of secondary contact. Both pre and post zygotic reproductive isolation are likely in an allopatric model of speciation since individuals with divergent habitat requirements are less likely to meet and mate. The hybrids produced by the matings between the divergent parents usually have a lower fitness status in their parental habitats (Templeton, 1981).

Habitat divergence may be potentially more important in sympatric speciation where reproductive barriers must evolve in the presence of gene flow. It is suggested that divergent selection leads to the rapid evolution of reproductive barriers (Felsenstein, 1981; Rice and Hostert, 1993). Habitat divergence could provide a valid means by which this might occur, because both prezygotic and post zygotic reproductive isolation are likely outcomes of adaptation to the local habitat.

1.3 Comparative studies and habitat adaptation

Comparative studies of plants grown under high and low levels of light have documented variation for several traits including leaf size and shape, dry mass per unit area, nutrient content, optical scattering and absorption, stomatal and trichome density, leaf mass area, and water use efficiency (Poorter and Garnier, 1999). The documented trait variation includes genetic as well as environmentally determined plastic responses to habitat variation.

Plastic responses are adaptive strategies on the part of the plant to cope with short-term environmental variation. For example, some traits such as leaf size as well as leaf mass area

exhibit phenotypic plasticity in response to their light environment (Bradshaw, 1965; Pigliucci, 2001). On the other hand, genetically determined variation is thought to be the result of adaptive evolution. It involves changes at allele frequencies and is related to fitness in different habitats (Hoffmann et al., 2002, 2005).

The documented genetic variation in leaf structure is largely adaptive for acquiring resources by photosynthesis (Gutschick, 1999). Photosynthesis is central to the plant's ability to compete and reproduce and is directly and dramatically influenced by the amount of light striking a plant's leaf surface as well as leaf's structural and biochemical differences. For example, variation in leaf size greatly affects the photosynthetic ability of a plant. Smaller leaves are considered to be more effective in dry habitats because a smaller boundary layer results in better heat exchange with the environment keeping the leaf at a better temperature for photosynthesis (Westoby and Wright, 2003; Hegazy and El Amry, 1998; Smith, 1978). At functional level, leaf traits such as stomata1 conductance, nitrogen content and chlorophyll content have been documented to affect photosynthesis (Bjorkman, 1981; Mooney and Gulmon, 1979). The effect of leaf structural variation is discussed in chapter 3 of this thesis.

Evidence for the adaptive value of key chemical, structural and physiological properties comes from studies which show them to be correlated across different habitats (Wright et al., 2004). Though the variation between habitats can be strongly marked this variation is all within the constraints imposed by various physiological, mechanical and developmental tradeoffs. The trait-trait correlations observed have provided evidence for three basic kinds of energetic tradeoffs that affect whole-plant rate of net carbon gain (Givnish, 1988). These involve the economics of gas exchange, economics of support, and the economics of biotic interactions (Givnish, 1988; Wright et al. 2004).

The economics of gas exchange arises from the link between uptake of carbon dixoide and loss of water both of which occur via the stomatal apertures present on the leaf surface (Givnish, 1986). Stomata allow the movement of large and slow CO_2 molecules into the leaf and the diffusion of smaller, faster water vapour molecules out of the leaf. The diffusion of smaller, faster water vapour molecules out of the leaf is essential for driving movement of water and minerals from the soil to the leaves. However, trade-offs exist between the leaf photosynthetic benefit of increasing the rate at which CO_2 can diffuse into the leaf against the transpirational benefits. The transpirational costs associated with increased CO_2 diffusion includes reduced photosynthetic capacity of the leaf due to low leaf water potential, reduction in the period of photosynthetic activity as well as increased allocation of energy to a well developed vascular systems as well as unproductive roots (Givnish, 1979; Beerling and Franks, 2010).

The tradeoff between photosynthetic benefits and leaf mechanical costs raises the economics of support. Plant species differ in the efficiency with which the leaves on the plant are mechanically supported (Givnish, 1986e). Leaf mass area (LMA) is a trait that measures the dry investment per unit of light intercepting leaf area deployed. Species with high LMA have thicker leaf blades or denser tissue or both (Lambers and Poorter, 1992. High LMA leaves represent high investment in structure. Such leaves are typically long-lived with lower photosynthetic rates (Wright et al., 2004).

Tradeoffs between photosynthetic benefits and biotic costs have generated the economics of biotic interactions. Herbivores are attracted to plants that are marked by erect growth habit and fast growth rate, high levels of nitrogen content per leaf, dense foliage to feed on, and low levels of defensive compounds (Gulmon and Mooney, 1986). Plants that experience herbivore and pathogen attack are reported to respond in a number of ways. One such

response is downregulation of photosynthetic genes to support the induction of a defence response. This has been observed in a number of studies (Delucia et al., 2010). Others such as the presence of trichomes and spines containing irritants and secondary metabolites such as nitrogen rich alkaloids are reported to deter herbivory damage. Divergence in leaf form could also be contributing to herbivore avoidance strategy (Givnish, 1984; Ehleringer et al., 1986).

The economics of gas exchange, support and biotic interactions are not only observed at the whole plant level but are also observed at the global level. This signifies the presence of a universal spectrum of leaf economics (Wright et al., 2004). The spectrum runs from quick to slow return on the investment of nutrients and dry mass in leaves. The spectrum reveals the linkage of high photosynthetic ability (A_{mass}) with high nitrogen content (N_{mass}), short leaf life span (LL), and low leaf mass area (LMA) (Wright et al., 2004).

Underlying the linkage between high photosynthetic ability (A_{mass}) and high nitrogen content (N_{mass}) is a direct causal relationship since nitrogen is a part the enzyme Rubisco which drives carbon fixation. In general, leaves with high photosynthetic ability require large inputs of nitrogen, phosphorus, and other mineral nutrients to create the pools of enzymes and pigments required (Field and Mooney, 1986).

The nitrogen rich leaves with high photosynthetic rates are also marked by short leaf life spans. The high nitrogen content and consequent high photosynthetic rates drive faster growth rates. Once the leaves attain maturity the plant sheds them transferring its resources to generating newer foliage (Givinish, 1986). The foliage in such plants possesses low leaf mass areas i.e., such leaves invest less per unit leaf area. Such leaves could be subjected to increased leaf vulnerability to herbivory and physical hazards, since they possess high protein content, low concentrations of lipids or lignin, and high concentrations of cheap constituents

(Villar et al., 2001). The correlation between high net assimilation rate and low leaf life span also reflect underlying adaptive constraints on plant trait evolution. For example, selection does not favor low net assimilation rate and short life span because such species would have low growth and low survival rates and would be eliminated (Wright et al., 2004).

1.4 QTL studies and habitat adaptation

Interspecific trait variation, comparative studies and patterns of trait variation found across communities, along resource and environmental gradients (light, water, nutrients, and temperature) strongly indicate a role of habitat divergence in plant speciation (Poorter and Garnier, 1999; Wright et al., 2004).

The variation between species can be used to explore the genetics of habitat adaptation (Orr, 2001). Underlying the adaptation to a specific habitat could either be a few alleles of large effect or many alleles of small effect. Large effect alleles become fixed more rapidly than those of small effects because of the large selective pressure driving fixation (Orr and Coyne, 1992). Based on whether a trait is controlled by large or small effect alleles, it can be predicted whether a phenotypic transition likely involved major leaps or occurred more smoothly (Burke et al., 2002).

Four or five genes of major effect and a few minor effect genes were responsible for the evolution of maize from *Teosinte* in contrast to the evolution of sunflower where many small effect genes played a part in the domestication process. This suggests a rapid evolution for maize and a smoother and gradual evolution for sunflower (Burke et al., 2002).

One of the many techniques used to explore the genetic basis of variation between different species is Quantitative Trait Locus (QTL) mapping (Mackay, 2001; Mauricio, 2001). To carry out QTL analysis, a genotyped segregating population is used to locate and characterise regions of the genetic map associated with variation in the population (QTL). A number of issues that are associated with the genetic basis of adaptation can be tackled with the help of QTL analysis such as the estimation of the number of loci contributing significantly to the population difference, as well as their effects, their interaction with each other and their interaction with the environment. Answering these issues is critical for the study of the genetic basis of adaptation.

Examples of model systems that have recently been developed for studying the genetic basis of adaptation in plants are *Mimulus* (monkey flowers), *Iris* (irises), and *Helianthus* (sunflowers).

Helianthus

Much evidence for habitat adaptation has come from studies conducted on the genus *Helianthus*. A number of traits reflecting adaptations were identified for three hybrid species found in three widely divergent habitats that interestingly shared the same parents *Helianthus annuus* and *Helianthus petiolaris* (Rieseberg, 1991). Rieseberg's study on the genetic architecture of leaf ecophysiological traits in early generation hybrids between *H. annus* and *H. petiolaris* revealed two QTLs for leaf nitrogen. The two QTLs detected in the study explained a quarter of the observed phenotypic variation (Rieseberg, 1991). A more or less similar study conducted by Ludwig et al. (2004) showed that hybrids with higher foliar nitrogen were significantly more successful in *H. anomalus* habitat. Since QTLs of large effect are expected to respond relatively quickly to selection, fixation of more fit genotypes having higher foliar nitrogen could proceed more quickly (Barton and Keightley, 2002).

Mimulus

Evidence for habitat adaptation in *Mimulus* also comes from a study conducted on two ecomorphs of *M. guttatus*. A reciprocal transplant experiment carried out on small, early flowering inland ecomorphs of *M. guttatus* and large, late-flowering *M. guttatus* ecomorphs of moist coastal habitats demonstrated strong selection for early flowering in the inland habitat and selection for later flowering at the coastal habitat. Hall and Willis (2006) highlighted the role of water availability in the selection process. They concluded that slow developing plants did not survive to flower at the inland site because of poor tolerance to summer drought (Hall and Willis, 2006). They further conducted QTL analyses in an F2 mapping population from a cross between coast and inland plants and resolved two pleiotropic QTLs of large effect that contribute 20–30% of divergence in multiple morphological and life-history traits between the ecomorphs, along with many additional loci of minor effect.

Differential adaptation of *M. lewisii* and its sister species *M. cardinalis* to different elevations that limit species ranges has also been tested and confirmed by reciprocal transplant experiment (Angert and Schemske, 2005). Ongoing research is examining the genetic basis of adaptations to different elevations. The prelimianary results suggest that it appears to be controlled by many loci (Wu et al., 2007).

Silene

Serpentine soils are rich in heavy metals such as nickel and cobalt. Studies conducted on two populations of *S. vulgaris* that grow parapatrically on serpentine and non-serpentine soil has provided evidence for habitat adaptation. The serpentine ecotypes of *Silene vulgaris* are characterized by dwarf stature, large root systems with faster root growth, small leathery leaves, as well as shorter internodes (Kruckeberg et al., 1990). Comparative experiments have

confirmed that adaptive traits in serpentine populations are genetically determined and not simply due to phenotypic plasticity (Bratteler et al., 2002). The genetic architecture of seven morphological, physiological and life history traits between serpentine and non-serpentine populations has revealed that most of the variation between traits potentially involved in habitat adaptation are controlled by a few genes of major effect.

1.5 *Begonia* as a study system for speciation

One of the main goals in the field of evolutionary biology is to understand the mechanism that leads to population divergence in the course of colonizing novel habitats, and to partition the contribution of genetic drift and natural selection (Wright, 1931). Genetic drift is a mechanism that is random and changes allele frequency but does not lead to adaptation. On the other hand, natural selection is a non-random process by which biological traits become more or less common in a population as they help individuals thrive in their environments. The tropics are highly biodiverse and provide ideal systems for investigating the processes underlying speciation (Emerson, 2008; Templeton, 2008). This study uses the large tropical genus *Begonia* to study the genetic and functional constraints underlying variation in vegetative form and function likely to be associated with adaptation to specific environments.

Genus *Begonia* is found throughout the wet tropics and is represented by over 1600 species (Doorenbos et al., 1998; Forrest and Hollingsworth 2003). The large size of the genus and its variation makes it ideal for studies of speciation (Neale et al., 2006; Brennan et al., 2012). At the genus level *Begonias* can be easily distinguished by asymmetry of leaf form, succulent petioles, unisexual flowers that are borne within the same inflorescence, and winged capsules (Doorenbos et al., 1998). Figure 1.1, illustrates some of the interesting features of the genus. At the genus level *Begonia* is divided into 66 sections. Among the 66 sections, 18 are Middle

American and include over 120 species. One such section includes *Begonia* section Gireoudia that consists of 66 species (Doorenbos et al., 1998, Burt-Utley, 1985).

Species in section Gireoudia are adapted to a huge range of habitats (Figure 1.2, Burt-Utley, 1985). The habitats in *Begonia* section Gireoudia vary in temperature and precipitation to a great extent. Mean annual temperature of regions where they have been collected (MAT) ranges from 21.7°C to 29.7°C whereas mean annual rainfall (MAR) ranges from 106 to 287.8 mm per year (Appendix 1.5, Nichola Harrison, unpublished).

The species in section Gireoudia are most abundant in wet forests throughout Mexico and Central America where they frequently colonise steep slopes and areas above streams. Species have also been reported growing in rainforests growing on tree trunks and rocks (Burt-Utley; 1985). Others such as *B. multinervia* are reported to grow on wet, open road banks and *B. heracleifolia* as well as *B. plebeja* are adapted to growing in seasonally deciduous forests throughout Central America and can tolerate high levels of temperature (Burt-Utley, 1985).

Species are characteristically rhizomatous perennials and exhibit tremendous diversity in shoot attributes and leaf form. The species in the section exhibits huge variation in leaf form and shoot attributes. The section seems to be cytologically homogeneous and consists of a diploid chromosome number of 2n=28 (Legro and Doorenbos, 1979; Dewitte et al., 2009; Brennan et al., 2012).

Most species in *Begonia* section Gireoudia form local populations ranging from a few to hundreds of individuals. Seed dispersal is poor as the seed have no dispersal agent and are not adapted for wind or water dispersal (Burt-Utley, 1985). Pollination is also poor and by

generalist pollinators (Burt-Utley, 1985). Low rates of gene flow between *Begonia* populations have been confirmed by a number of population genetic studies (Hughes et al., 2003). This also holds true for the species in section Gireoudia (Twyford, 2012; Twyford thesis). Despite the wide distribution of some of the species including *B. sericoneura* and *B. heracleifolia* most of the species in this section are fairly narrow endemics, as is typical for *Begonia* (Burt-Utley, 1985, Dewitte et al., 2012).

Limited seed dispersal and limited gene flow among populations, resulting in a low effective population size in this section might have aided selection driving phenotypic divergence. Additionally, it could also have resulted in a strong role for genetic drift in the production of variation between species. However, random fluctuations in trait means created purely by genetic drift are unlikely to have produced coherent suites of traits observed in a range of species.

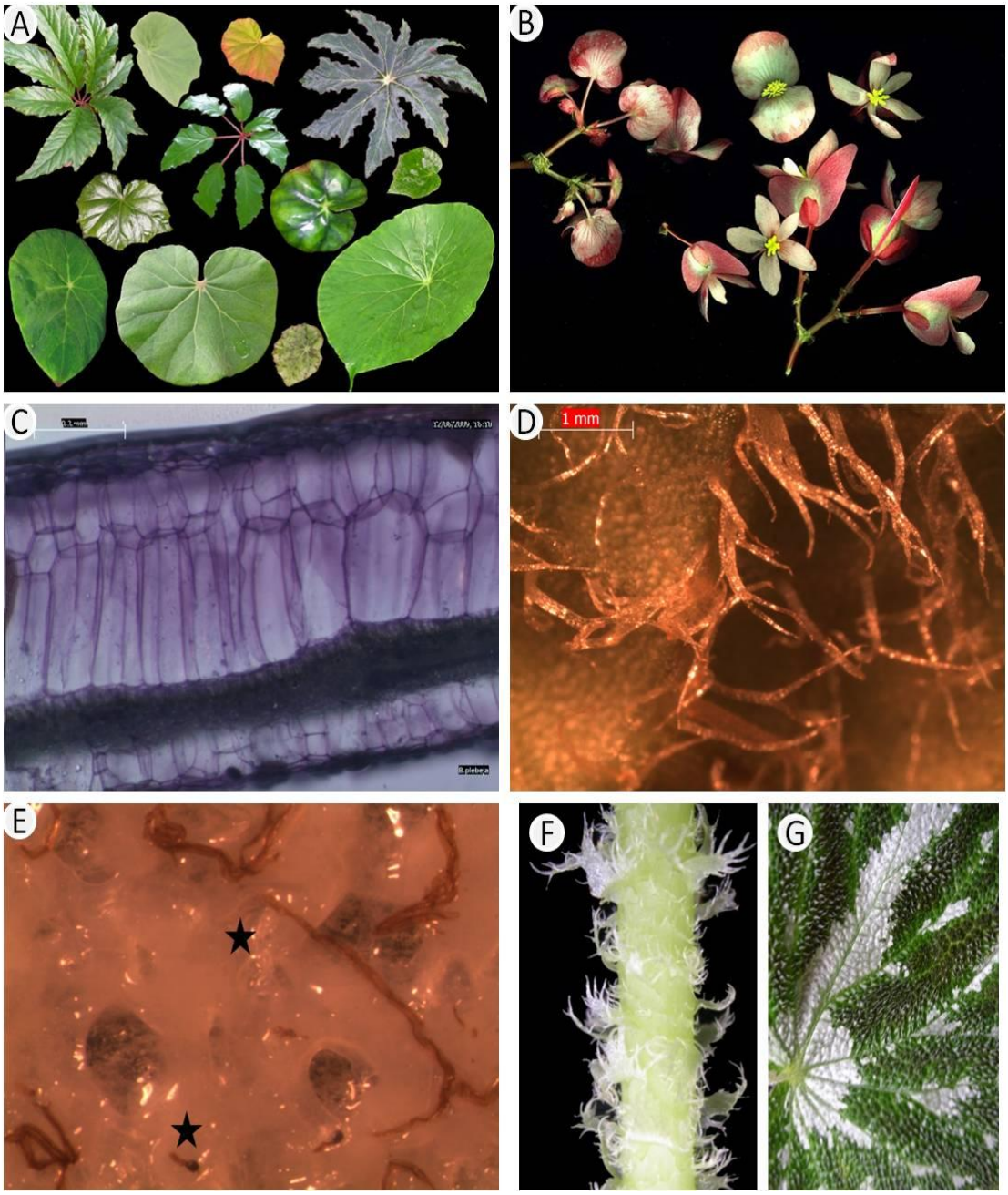

Figure 1.1: Some of the interesting traits in the genus *Begonia.* (A) *Begonias* exhibit tremendous diversity in general leaf size and shape; *Begonias* are often large and variously marked or variegated and are usually asymmetric. Both simple and compound leaf species are present in the genus (B) *Begonias* are characterised by unisexual flowers that are borne within the same inflorescence, and possess winged capsules (C) *Begonias* exhibit tremendous diversity in the internal leaf anatomy (D) Scales and villi present on *Begonias* (E). Whiplash and glandular trichomes present on *B. conchifolia* leaf (F) Petiolar induments observed for some of the species (G) Epidermal elaboration.

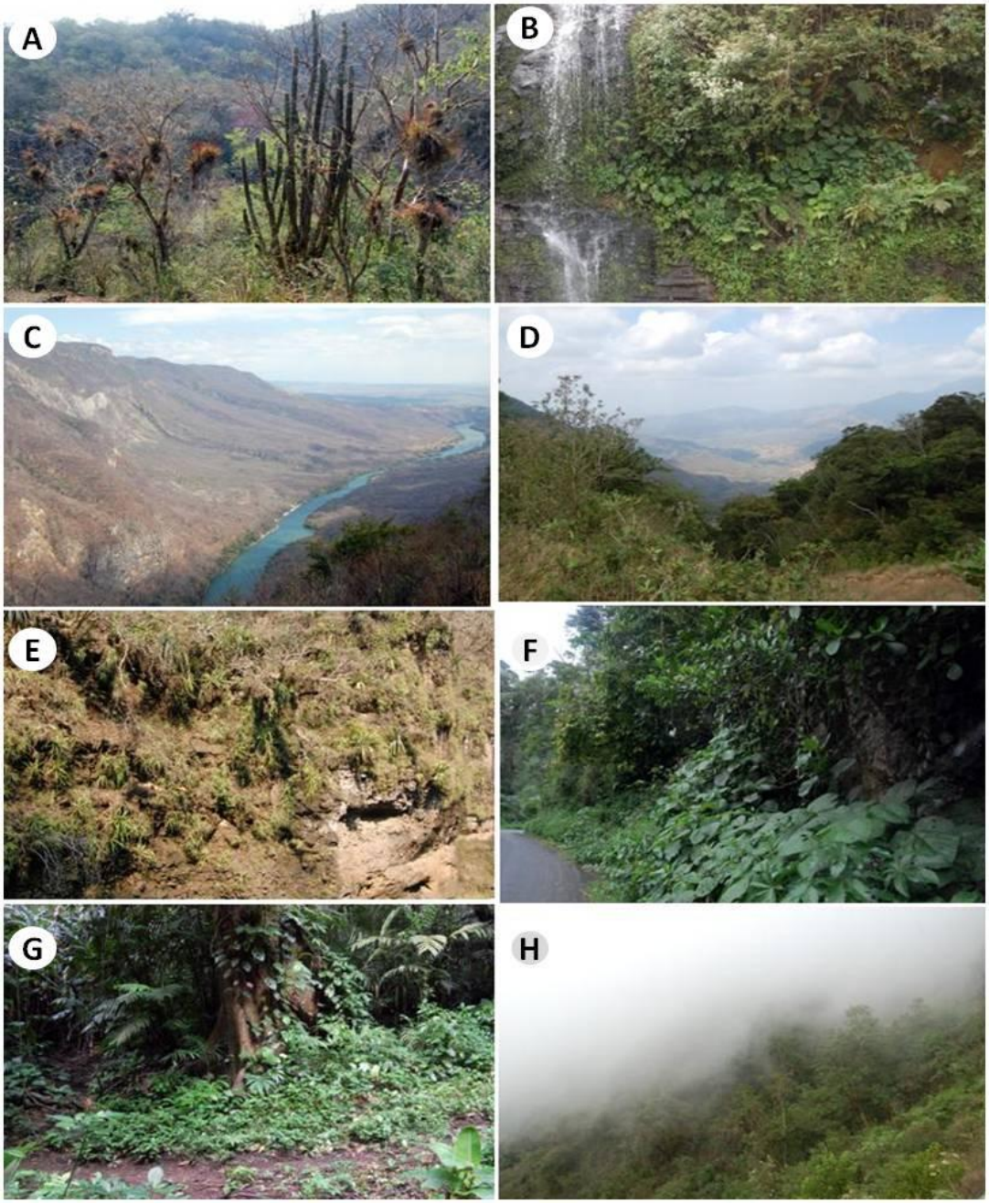

Figure 1.2: Variation in *Begonia* habitats in Southern Mexico. (A) canyon near Coyol Veracruz, habitat of *B. peltata*, (B) *B. multistaminea* growing in abundance near a waterfall (C) Sumidero Canyon Chiapas, surrounded by seasonally dry forest (D) degraded tropical rainforest (E) canyon near Calchualco Veracruz, locality with abundant drought adapted *B. hydrocotyfolia*, (F) roadsides frequently have a number of weedy *Begonia* species such as *B. heracleifolia, B. nelumbiifolia* (G) primary rainforest at union Juarez (H) transitional zone between dry forest and cloud forest sees a clear demarcation in species, *B. fusca* grows nearby. These pictures were taken by Alex Twyford during his field trip to Mexico.

1.6. Morphology, habitat and distribution of *B. plebeja* and *B. conchifolia*

To dissect the genetic architecture of species-level variation in detail we selected two species namely *B. plebeja* and *B. conchifolia*. These are closely related (Nichola Harrison PhD thesis 2012) but found in very different habitats (Nichola Harrison unpublished, Burt-Utley, 1985), and are very distinct morphologically.

Morphology

B. conchifolia is distinctive in the section and in the genus as a whole due to its small succulent, persistent, peltate leaves. It is a repent species, creeping along rocks or tree trucks with thin, highly branched rhizomes. On the other hand *B. plebeja* has large, flat thin leaves with a typical '*Begonia*' asymmetric shape and a robust upright growth habit (Figure 1.3)

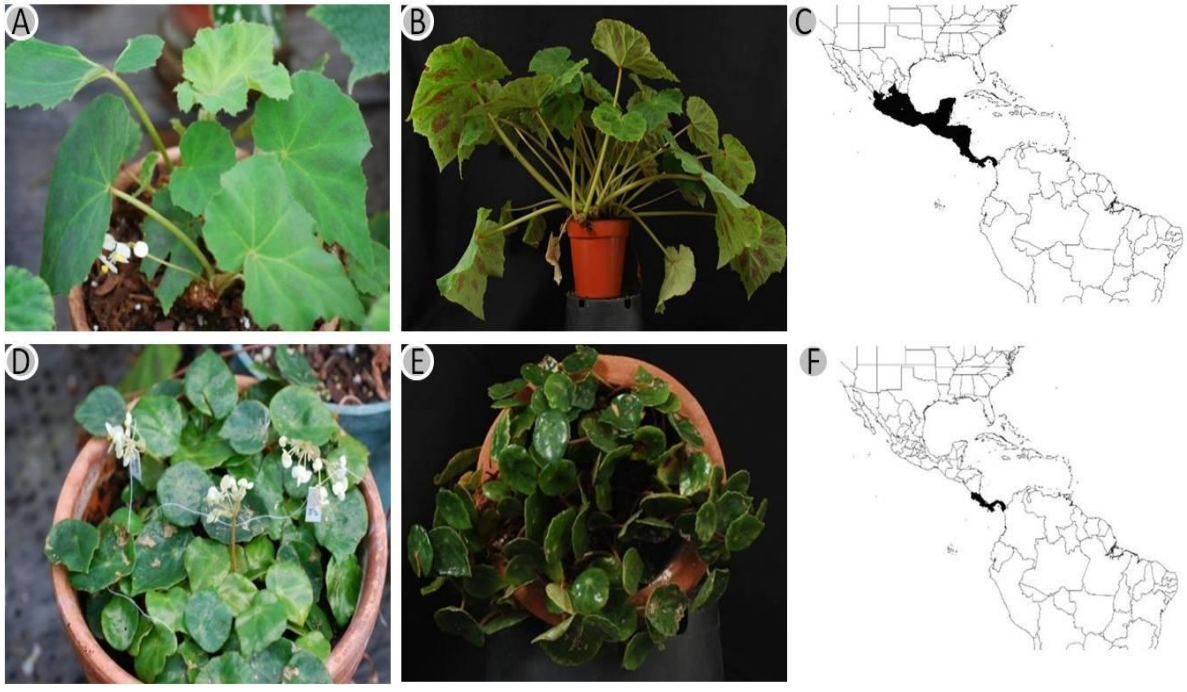

Figure 1.3: *B. plebeja* (A, B) is found in Mexico and throughout Central America (C) *B. conchifolia* (D, E) is an inhabitant of wet rain forests in Costa Rica and Panama (F) Bars: (A–U) = 0.2 mm.

Habitat

B. conchifolia is often found on lower portions of tree trunks and is epiphytic on steep rock faces where other vascular plants have not yet established. *B. plebeja* is a frequent inhabitant of tropical deciduous forests where it often grows epiphytically or saxicolously in exposed locations. Populations in seasonally dry areas like the Valle Central in Costa Rica are deciduous, but populations from areas where the environment is less harsh may show variation in this character (Burt-Utley, 1985).

Distribution

B. conchifolia occurs in wet rain forests in Costa Rica and Panama between 550-2000m elevations where they form small, highly local populations whereas *B. plebeja* is much more widespread, from Central Mexico to central Panama between 40m to 1500m elevation (Burt-Utley, 1985).

1.7 Questions addressed by the project

Divergent natural selection has promoted speciation in a wide range of taxa. Our aim is to investigate habitat driven divergent selection in *Begonia* section Gireoudia. Section Gireoudia is a recent radiation into a highly diverse environment of Mexico (Nicola Harrison, PhD thesis 2012 that is recognised for its species richness and biodiversity (Myers et al., 2000). Mexico was likely colonised by *Begonias* adapted to the wet montane rainforests of South America (Harrison 2012 PhD thesis; Goodall-Copestake 2011). In Mexico *Begonias* found a wide range of habitats, including some very much more open and drier than those they would have come from. There are now a number of species endemic to mesoAmerica including some adapted to habitats much drier than usual for *Begonia*s such as *B. heracleifolia* and *B. plebeja.* I expect adaptation of these species to a drier environment to

have involved a suite of morphological and physiological changes in plant form, cellular anatomy, physiology and resource allocation and growth patterns.

To understand how some species in this section may be adapted to new environments I first created an overview of the variation within the section as a whole for a range of morphological, ecophysiological and anatomical traits (Chapter 3). This creates a picture of the 'trait space' which section Gireoudia occupies and identifies correlated suites of traits. I then examine the significance of trait differences between two species found in divergent habitats to identify adaptation to light and shade environments (Chapter 4). The genetics underlying these traits is examined in Chapter 5, using an F1BC1 hybrid population. I use the variation in the mapping population and in the section as a whole to examine evidence for selection of suites of traits as an adaptive response to the challenges of the new habitats in Chapter 6. Chapter 7 presents a QTL analysis of the F1BC1 hybrid population to determine whether the genetic architecture of these traits suggests they could easily become fixed between populations and look for genetic linkage of functionally correlated traits.

Hypothesis:

If Section Gireoudia speciated through habitat adaptation to specific environments:

1. Species will differ significantly in ecophysiology and in morphological traits associated with that ecophysiology (Variation with the section as a whole is described in Chapter 3. Chapter 4 looks in greater detail at a closely related pair of species found in contrasting environments, associations examined in Chapter 6).

2. The genetic complexity of these traits will reflect how easy they are to evolve (Chapter 5 describes the segregation patterns of traits in a hybrid population between the two contrasting species).

3. There may be correlations between traits associated with adaptation to specific environments (Chapter 6 examines correlations between traits in the section as a whole and in the hybrid population in the framework of what has been described for plants globally).

4. Does the genetic architecture of the variation between species suggest that adaptative changes could happen quickly (Chapter 7 describes a QTL analysis of variation in the hybrid population).

CHAPTER 2.Materials and Methods

2.1 Plant Material

2.1.1. Species of Section Gireoudia used in the project

A total of 21 species from section Gireoudia were included in this project (Figure 2.1). The species were grown in the glass houses of Royal Botanic Garden Edinburgh in tropical climatic conditions (approximate day temperature 28˚C, night temperature of 20˚C and a relative humidity of 70%). Table 2.1 lists all the species used in this project.

Table 2.1: Enlists a detailed list for some of the species in *Begonia* section Gireoudia. The species were used in the project.

List of species	Distribution	Accession no
B. plebeja	Mexico & S. America	20051406 / 1015.95
B. conchifolia	Central America	20042082
B. nelumbifolia	Mexico, Guatemala, Columbia	19791880
B. cardiocarpa	Central America	20051398 / GL00500692
B. stigmosa	Mexico	20051413 / GL00807986
B. mazae	Mexico	19551057/ GL002-045-04
B. squarrosa	Mexico	AT 1075 / 20071058
B. kellermani	Guatemala	20030642
B. multinervia	Central America	20051411/ GL01404489
B. sericoneura	Mexico	20051412/ GL019-123-70
B. peltata	Mexico, Guatemala	2004078
B. corredorana	Costa Rica	20071055
B. hydrocotilifolia	Mexico	20030641
B. puriniata	Costa Rica	GL040-103-83
B. carriae	Mexico	20051408
B. carolinefolia	Mexico	20042077/ GL00108299
B.heracleifolia	Central America	20042080
B. multistaminae	Mexico	20071056
B. involucrata	Central America	GL004-100-57
B. thiemie	Mexico	20042079/ GL00209379
B. lindiliyana	Guatemala	20051412/ GL01312370

Figure 2.1: Species in *Begonia* section Gireoudia growing in the green house in Royal Botanic Garden Edinburgh. These include *B. carriae* (A), *B. multinervia* (B), *B. plebeja* (C), *B. corredorana* (D), *B. peltata* (E), *B. heracleifolia* (F), *B. hydrocotilifolia* (G), *B. nelumbifolia* (H), *B. thiemie* (I), *B. Stigmosa* (J), *B. conchifolia* (K), *B. mazae* (L), *B.kellermani* (M), *B. purianata* (N), *B. carilinefolia* (O), *B. cardiocarpa* (P), *B. lindliyana* (Q), *B. puriniata* (R), *B. multistaminae* (S), *B. sericoneura* (T), *B. squarrosa* (U).

2.1.2 Construction of backcross populations

For the generation of a genetic map two species from section Gireoudia were selected – *B. plebeja* and *B. conchifolia*. The species are found in different habitats in Central America and Mexico (Burt-Utely, 1985). An F1 hybrid generation of eight plants was produced in 2006 (numbered CKB 137.1 to CKB137.8). One of the F1 hybrids, CKB137. 8, was then used as a pollen recipient to the *B. plebeja* parent, to generate the backcross population (B08-360) of 400 plants in 2009 (Figure 2.2). B08-360 was used for the generation of a genetic map and for genetic analysis of a range of traits.

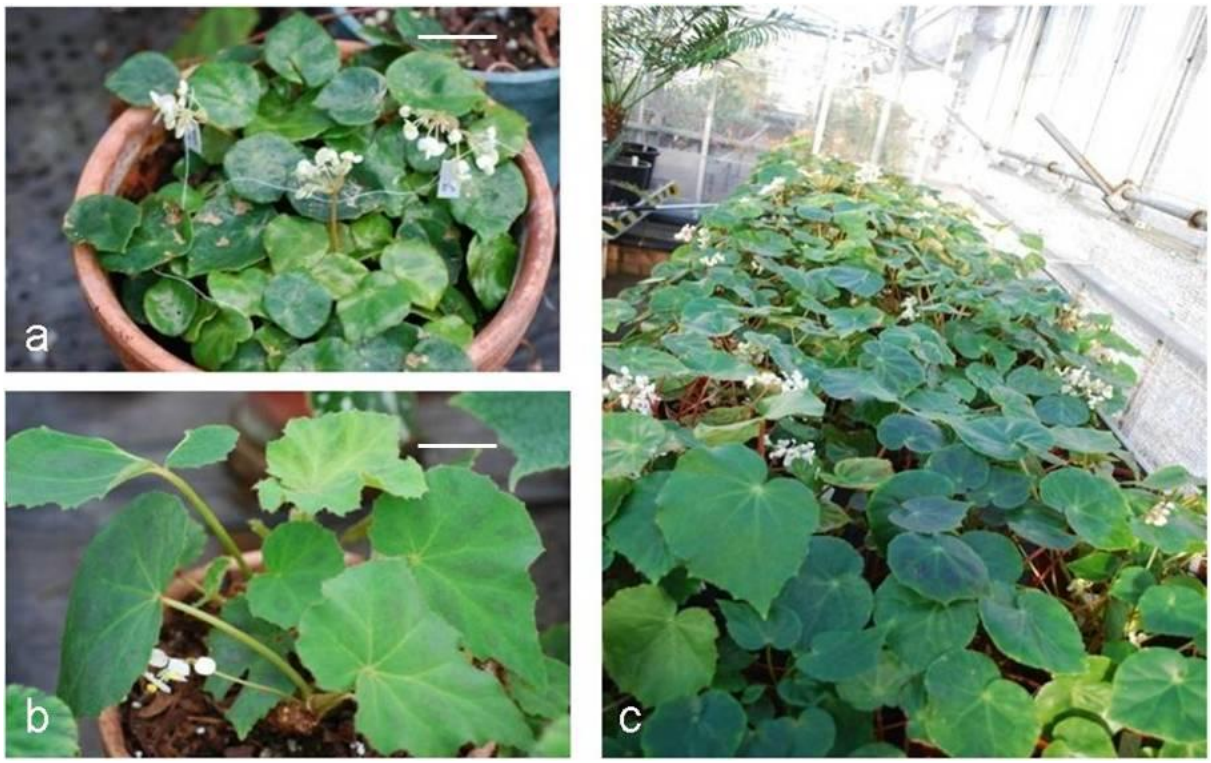

Figure 2.2: *B. conchifolia* (a) *B. plebeja* (b) and the mapping population (c) growing in the glass house in Royal Botanic Garden Edinburgh.

<u>Germination and plant growth</u>

A protocol (unpublished) was obtained from Angelo de Witte (A PhD student at ILVO Horticultural Research Institute, Ghent, Belgium) and was used with little modification to

germinate B08-360. Fifty seeds were grown per plate. Circles of Miracloth with a diameter slightly smaller than a Petri dish were cut and were put in a foil for autoclaving. A bottle of sterile water and 1 litre media solution were prepared. The media solution contained 1.46 g/l MS salts, and 20 g/l sucrose. Its pH was then adjusted to 6.2 with KOH and HCl. Then 5 g/l Phytogel or agarose were added to set the plates. The plates were then autoclaved. A sterilization solution of 10% bleach and 0.005% Teepol (or Tween or Triton-X) were then prepared. Meanwhile media solution was cooled to 50˚C. After cooling 1ml/litre of solution of previously filter sterilised was added to that. The filter sterilised solution contained 100mg/l meso inositol; 1mg/l nicotinic acid; 1mg/l glycine; 1mg/l GA3; 0.5mg/l thiamine; and 0.5mg/l pyridoxine. Plates were then poured in a laminar flow hood under sterile conditions and were then left to set in flow hood (lids off) for 1 hour (or overnight). Next, plating of seeds was carried out in flow hood. The flow hood was turned off for this step. Fifty seeds per batch were then put onto Miracloth circles just smaller than the diameter of a petri dish. The Miracloth was folded to a wedge shape and held in place with paperclips. Miracloth packages were put into Falcon tube and were covered with a sterilization solution of freshly mixed 10% bleach and 0.005% Teepol. Tubes were lightly inverted for a few times and were then left for 20 minutes. The packages were rinsed 5X with sterile water. Miracloth packages were then opened with sterile tweezers and were placed face down onto media plates. Plates were then kept for germination at 24-28˚C under a 16 h photoperiod (40 μmol $m^{-2}s^{-1}$). After germination seedlings from plates were transferred to sieved tropical potting mix. The tropical potting mix consisted of a mixture of coarse, medium and fine bark along with John Innes no 1, a slow release fertilizer (Osmocote) and perlite which helps in drainage. Seedlings were allowed to acclimatize to the open air on a warm spray bench for one month. They were then potted on into growing pods in unsieved tropical mix.

2.2 Phenotypic scoring of traits

All plants were grown in the glasshouse under the same growing conditions in tropical climatic conditions (approximate day temperature 28˚C, night temperature of 20˚C and a relative humidity of 70%). Each species accession was represented by two plants in the survey. For micromorphological traits in the survey three fields of views for each leaf were taken for each trait under the light microscope. Average of the values was used as the final reading. The number of replicates used for *B. plebeja* and *B. conchifolia* varied in the survey since it was difficult to take measurements on small *B. conchifolia* leaf. Data was harvested on one leaf per plant on the individuals in the mapping population. Given below is a description of the traits measured and the codes used in the study.

2.2.1. Morphological traits

Morphological data was gathered on a set of discrete and continuous traits which may be involved in conferring an adaptive value in terms of fitness as well as on traits which were clearly variable in parent and backcross populations.

❖ **Anthocyanin pigmentation**

The anthocyanin pigmentation was scored visually on stipules, rhizomes, petioles and leaves.

a. Anthocyanin pigmentation on stipules

Stipule colour was scored as shades of red and was categorised in eight categories. The codes are as follows. 0=No red colouration, 1= v weak red colouration, 2= weak red colouration, 3= medium red colouration, 4= medium-strong red colouration and 5= Strong red colouration.

b. Anthocyanin pigmentation on rhizome

The colour of rhizomes was very variable and probably was under very strong environmental effect of shading. Only the colour of the upper surface of the rhizome was scored in this study. The colour of rhizome was scored as no red pigment ("g" as green) or presence of red pigment to any extent ("r" as red). In the data set 'g' was coded as 1 and "r" was coded as 0.

c. Anthocyanin pigmentation on petioles

The colour of petioles was scored as shades of red, in the main petiole and in the majority of younger petioles**.** Four categories were used to express anthocyanin pigmentation on petioles including 0= no red colouration, 1 = weak red colouration, 2 = medium red colouration and 3 = strong red colouration.

d. Anthocyanin pigmentation on leaf blade (Extent, form, intensity)

The presence of anthocyanins is referred to as "leaf blotches" in this study. Leaf blotches are a very distinctive trait and the size and intensity of the blotches varies widely. Leaf blotches have been categorised as presence/absence, distinctive size (large or small) or intensity (weak or strong) variants.

e. Anthocyanin pigmentation on petiole attachment point (Red eye)

The point at which the petiole is attached to the lamina is referred to as the eye in this study. The presence of "red eye" has been coded as 0 =Yes and 1= No. The extent of expression of anthocyanins in the red eye region were coded as 0 = white, 1= Pink, 2 = Red, and 3 = Dark red.

f. Anthocyanin pigmentation on leaf margins

The extent of expression of anthocyanin on leaf margins was coded as weak = 1, red =2, dark red = 3.

❖ Plant architecture

Variation is observed between Gireoudia species for size and architectural characters. Some of the traits that could help us differentiate between compact and spreading architectures were measured and are described below. The information was obtained using vernier callipers, measuring tapes, and Lamina software. Lamina is free software and is available at http://sourceforge.net/projects/lamina (Bylesjo et al., 2008).

a. Total number of rhizomes

The main and axillary rhizomes were together scored as total number of rhizomes on the plant.

b. Length / width of rhizomes

The total length and width of each rhizome was measured with measuring tapes and vernier callipers.

c. Total leaves per cm of rhizome

The total number of leaves produced per mm of rhizome was also scored in the study.

d. Area and leaf shape parameters

Basic leaf dimension parameters such as area, length, and width as well as measures of leaf shape, symmetry, serration number, depth and the missing area within a leaf were quantified using the Lamina programme (Bylesjo et al., 2008). Areas of leaf and shape measurements were taken when the plants were one and a half year old.

2.2.2 Measurement of functional Traits

a. Photosynthesis and stomatal conductance

The system used to measure the photosynthetic rates of the *Begonia* leaves was the LI-COR-6400 Portable photosynthesis system (LI-COR Inc., Lincoln, US). LI-COR-6400 Portable photosynthesis system is an open system which measures the photosynthesis and stomatal conductance of the leaf area that is contained within a 6 cm^2 leaf chamber (LI-COR, 1999). The important units of the system comprise of desiccant (drierite), soda lime (5% NaOH, 1% KOH, 0.2% silica, 14-19% water and Ca(OH)2 to make a total of 100%) and a CO_2 cylinder. The desiccant (drierite) scrubs water from the incoming airstream that flows into the chamber allowing humidity to be controlled, whereas soda lime (carbosorb) scrubs CO_2 from the air. In order to provide a stable CO_2 concentration at 380 ppm, a 12 gram CO_2 cylinder was used in the CO_2 mixer to inject enough CO_2. The experimental conditions required ambient relative humidity and a saturating light intensity for photosynthesis i.e, 700 µmol m^{-2} s^{-1}. To achieve ambient relative humidity, the desiccant tube adjustment knob was set between scrub and bypass meaning that not all water was removed from the incoming air. The red and blue light emitting diode lamp that allowed a constant quantum flux to be produced within the leaf chamber was set at the saturating light intensity of photosynthesis. Since the light saturation points varied for *B. plebeja* and *B. conchifolia* as well as the F1BC1 hybrid it was decided to use an average of all the saturation points. In the light of this a saturation point of 700 µmol m^{-2} s^{-1} was used to measure the net assimilation rate for the rest of Gireoudias and the plants in the mapping population. The flow rate of air through the chamber was set at 500 µ mol s^{-1}.

An external quantum sensor mounted on the sensor head recorded the light conditions for the plant outside the leaf chamber. The air and leaf temperatures were recorded using a linearised thermistor inside the sample cell (in the sensor head) and a thermocouple in the leaf chamber

respectively. Once the leaf was stable, it was held in the leaf chamber to record any change in the CO_2 and H_2O. The changes in the CO_2 and H_2O were recorded using sample and reference infra red gas analysers (IRGAs) in the sensor head.

2.2.3 Measurement of chemical traits

The traits analyzed in this category are chlorophyll content (spad), nitrogen (%) and carbon content in percentage (%).

a. Chlorophyll content

The chlorophyll content of the leaves was measured using a Minolta SPAD-502 (Soil Plant Analysis Development) meter (Spectrum Technologies, Illinois, US). The spad meter gives a 'SPAD' unit which can be converted to chlorophyll concentration. The spad meter determines the relative amount of chlorophyll in the leaves by measuring the transmittance of the leaf in the red and infra red regions at 650 to 940 nm. Three meter readings were carried out on the same leaf used for the photosynthesis measurements. These were averaged to give a single mean reading for each leaf.

b. Nitrogen and carbon content

The leaves used for the photosynthesis and chlorophyll measurements were removed and placed into labeled paper bags. They were then weighed. After this the leaves were dried in the oven at 105°C until constant weight was reached.The leaves were then ground to a fine powder using a ball mill. The sample (one leaf) was placed in a ball mill, ball bearings were added and the container clamped into place. A motor then shook the sample vigorously for 5 minutes.

The samples were then prepared to be analyzed by a Carlo Erba NA2500. Between 3 mg and 4 mg of dried, ground sample material was accurately weighed into a tin capsule. The samples were then introduced into the combustion tube, (which is maintained at about 1000°C via an auto-sampler). This was done by Ann Mennim, Technical Support Officer in the School of Geosciences, at the University of Edinburgh.

2.2.4 Measurement of resource use strategy traits

a. Leaf mass area

The LMA of the leaves was calculated by dividing the leaf dry weight (g) into the leaf area (m^2) to give units of g m^{-2}.

b. Leaf dry matter content

Leaf dry matter content was determined by dividing dry weight of leaf by leaf fresh weight (mg g^{-1}).

2.2.5 Measurement of micromorphological traits

Twenty seven foliar characters were captured during the micromorphological survey. For capturing micromorphological characters we used several different methods. All observations were made using fresh mature leaf material. Three fields of views were taken for each trait under the light microscope. The mean of the values was used as the final reading.

a. Transverse leaf sections

Sampling was carried out in between secondary venation in the middle part of the lamina for microscopic observation. Fresh transverse sections were done free hand using a razor blade. Transverse leaf sections gave sixteen traits. The traits were width of lamina, width of vein,

depth of adaxial epidermis cell layer, depth of adaxial hypoderm layer, width of an adaxial cell, number of adaxial hypoderm cells, type of adaxial hypoderm cells, depth of abaxial epidermis cell layer, depth of abaxial hypoderm layer, width of an abaxial cell, number of abaxial hypoderm cells, type of abaxial hypoderm cells, depth of mesophyll, mesophyll structured (pallisade and spongy mesophyll division), depth of palisade mesophyll, depth of spongy mesophyll and anthocyanins in parenchyma.

b. Epidermal flap (paradermal leaf section)

Sampling was carried out in between secondary venation in the middle part of the lamina. A number of traits were analyzed using paradermal sectioning. These included density of stomatal clusters, number of stomata per cluster, distance between clusters, substomatal cavity width, substomal cavity length, number of stomata in each cluster, and arrangement of stomata.

c. Scanning electron microscopy

To score the developmental process of stomatal clusters in *Begonia* section Gireoudia**,** **e**xpanding leaves that had stomatal clusters of varying developmental ages on them were selected from *B. sericoneura.* To study stomatal cluster development the leaves were fixed in FAA and critical point dried. The material was visualised with a Leo Supra 55 VP scanning electron microscope (SEM). Measurements were taken from the abaxial leaf (lower) surface.

Critical point drying method using FAA

For SEM, the specimen chamber is at high vacuum and therefore requires a completely dry sample. For this purpose leaves were chemically fixed to preserve and stabilize their structure. Fixation was performed by incubation in a solution of a buffered chemical fixative, formaldehyde acetic acid alcohol (FAA) formulation. The sample was then fixed overnight in

FAA at room temperature. The next day, the samples were then thoroughly rinsed several times in 50% ethanol to remove FAA. Once the FAA was removed, the samples were prepared for drying. In the drying process the water in the cells was replaced with ethanol then acetone. It was processed through 70% ethanol for 15 min, 95% ethanol for 10 min, then rinsed with 100% ethanol for 5 minutes and 100% acetone for 5 min. Ethanol and acetone were then replaced at pressure with liquid carbon dioxide. For that the samples were transferred into the pre cooled CPD (critical point dryer) taking care not to let the specimens dry out. Once the specimens were processes the baskets were moved to desiccant as quickly as possible. The highly hydroscopic and transferred specimens were then mounted and sputter coated without delay to reduce the risk of damage. Image analysis was then carried out with Leo Supra 55 VP scanning electron microscope (SEM).

d. Petiole transverse section

Free hand transverse sections of fresh material were used for analysis of petiole anatomy. The vascular bundles could be clearly grouped into small and large bundles. Width of petiole cross section, number of large inner vascular bundles, number of small outer vascular bundles, length of large, width of large vascular bundles, length of small vascular bundle and width of small vascular bundle were measured in this category.

e. Sticky tape sections

Sticky tape sections coated with an adhesive material were used to remove trichomes from the abaxial and adaxial sides of the leaf. The tape sections were then examined under the microscope to gather information on type of trichomes, length of trichomes and number of trichomes per mm^2 for the abaxial and adaxial sides of leaf.

2.3 Generation of genetic map and QTL mapping

Transcriptome analysis and the generation of the genetic map were carried out as described in Brennan et al., 2012 (paper attached at the end of thesis).

2.4 QTL mapping

The genetic map was used for QTL analysis of traits that differed between species including morphological traits, leaf form, ecophysiological traits, and micromorphological traits. QGene software was used for the purpose of QTL mapping. For analysing the binary (either nominal or ordinal) data, I carried out a series of contingency tables and chi- squared tests for independence of trait scores tabulated against the genotypes at each marker in turn, with markers in map order. Ordinal traits having a maximum of four categories were analysed with the help of a MIM-GLZ plug-in based on Generalized Linear Model (GLZ) in Qgene. Ordinal traits with more than six categories were treated as continuous traits for QTL mapping (ordinal with more than 2 categories). For analysing the continuous trait data (count and metric), composite interval mapping CIM (Zeng, 1994) was first carried out in QGENE (Nelson, 1997). The LOD scores were calculated from the CIM results. Genome-wide threshold LOD values to declare a QTL to be significant were determined with 500 permutations for each trait as implemented in Qgene. A QTL was defined as a major one when the percentage of variance explained (PVE) was over 25%. Directions of QTL effects (plus or minus) for each parental map were also calculated for each trait. For non-normally distributed trait data the results were confirmed with non-parametric method based on MIM-GLZ based on the Generalized Linear Model (GLZ) in Qgene.

2.5 Statistical analysis

The values for each leaf trait were averaged to determine the mean trait values per plant. Univariate statistics, correlation analysis, principal component analysis and cluster analysis

was carried out on the average trait values. For univariate statistic and the generation of histograms for the species in section Gireoudia and the individuals in the mapping population PAST software was used (Hammer et al., 2001). Simple correlations between morphological, ecophysiological and micromorphological traits were analyzed using Spearman rank order correlations in R software. Correlation coefficients were determined using codes in R software. The codes was written by Tobias Marczewski listed (with his permission) in appendix section. Principle component analysis and the Hierarchical cluster analysis based on Ward's (1963) method were carried out in PAST software.

CHAPTER 3.Trait variation in *Begonia* section Gireoudia

3.1 Brief overview

Adaptations are features that allow plant species to survive and compete successfully in a particular habitat. Adaptations to a particular habitat may include morphological, ecophysiological and micromorphological traits. Documenting plant trait variation and understanding exactly how various morphological and physiological properties help a plant compete in certain environments but not in others has permitted us to understand patterns in the distribution of species.

Net assimilation rate

A well studied measure of how well a plant is performing in its habitat is light saturated net photosynthesis (A_{max}), the maximal rate of photosynthesis minus the rate of respiration A_{max} vary greatly amongst plant species, from nearly 0 to nearly 30 μmol m^{-2} s^{-1} (Larcher 2003). Differences in A_{max} are accompanied by differences in morphological and functional attributes such as leaf morphology, internal leaf anatomy, epidermal structures, draw down of CO_2 inside the leaf, stomatal conductance and physiological variation (Wright et al., 2003). Much of the variation in leaf structure (at both microscopic and macroscopic level) and function seems to be driven by an effort to compete successfully in new environments and to maximise photosynthesis.

At the leaf functional level, many traits including stomata1 conductance, leaf nitrogen content, and chlorophyll content are known to be correlated with total gas exchange (Bjorkman, 1981; Mooney and Gulmon, 1979).

Stomatal conductance plays a vital role in water exchange between plant and atmosphere and is therefore a key parameter in many ecological models (Chen et al., 1999). Mainly driven by stomatal aperture, the diffusion of CO_2 into the mesophyll and water vapour from the leaves to the atmosphere is controlled by a complex system of plant physiological processes. This system varies to a great extent among species and taxa. The stomatal conductance is greatest in leaves of herbaceous plants and particularly low in woody plants with thick and stiff leaves (Larcher, 2003).

Also integral to the process of carbon assimilation is leaf nitrogen content. Nitrogen rich enzymes such as Rubisco are integral to photosynthesis (Field and Mooney, 1986). Leaf nitrogen is among the six key leaf traits that together capture many essentials of leaf economics at the global level. The linkage of high A_{max} with high N_{mass} is in large part the result of a direct relationship and has been observed at the global level in 2,548 species from 219 families at 175 sites (Wright et al., 2004).

Chlorophyll content also influences the net assimilation rate and varies to a great extent between species and taxa (from 1 up to nearly 1000 μmol m^{-2}). Chlorophyll is required for the absorption of solar radiation. If the chlorophyll content is low it can adversely affect the photochemical processes and hence the overall photosynthetic rates. Chlorophyll content is used in breeding programs as an effective index of high photosynthetic efficiency (Kannangara, 1991).

The drawdown of CO_2 inside the leaf is also affected by the structure of the leaf. Leaf mass area (LMA) measures the dry investment per unit of light intercepting leaf area deployed. High LMA species contain high concentrations of lignin and other secondary compounds in their leaves and possess thicker leaf blades or denser tissue or both (Lambers and Poorter,

1992). Such leaves are also marked by low concentrations of nitrogen, proteins & minerals and hence results in low rates of photosynthesis (Poorter et al., 2009).

Leaf carbon content is a more specific measure and assesses investment in carbon-rich structural molecules such as cellulose. It correlates with the construction cost of leaves (Nagel et al., 2002). At a structural level traits such as the effective size of the leaf (measured by using diameter of the largest circle drawn inside the leaf surface) (Givnish and Vermeij, 1976), shape of the leaf (Givnish, 1984), orientation of the leaf (Nobel, 1986, Vos et al., 2010) and internal leaf anatomy (Parkhurst, 1986, Kaldenhoff, 2012) have been shown to affect net assimilation rates.

Leaf size and shape

There is ample evidence that leaf sizes and shapes have evolved to maximise returns by performing the main function of gathering energy from sunlight, and exchanging carbon dioxide and oxygen. The significance of leaf size variation has also been looked at from thermal balance perspectives (Westoby and Wright, 2003).

Leaf size varies greatly between, and sometimes within species (Sack et al., 2006). There is also strong evidence that leaf sizes vary with the environment (McDonald et al., 2003). In general, leaf size decreases with increasing altitude, decreasing rainfall and soil nutrient content. Large leaves are usually associated with wet places and tropical rain forests whereas small leaves are associated with dry places as an adaptation to conserve water. Large leaves are also considered to be more economical from a mechanical point of view compared to small leaves. It is because large leaves require a less elaborate support system than many small leaves that they may be favored under certain conditions (Givinish, 1979).

From thermal balance perspectives, smaller sized leaves are considered to be more effective in dry habitats because smaller leaves possess smaller boundary layers and better heat exchange with the environment (Westoby and Wright, 2003).

Variability for leaf shape has been documented in many taxa and ranges from minimal to tremendous (Hay and Tsiantis, 2006, Kidner and Umbreen 2010). The genus *Protea* exhibits minimal leaf shape diversity whereas species of the family Papaveraceae and the genus *Pelargonium* exhibit tremendous variation (Jones et al., 2009). Plants in sunny habitats are characterised by leaves that are smaller and have complex edges and lobes whereas leaves in the shade tend to be larger and fewer number of lobes and edges. Smaller leaves have a reduced light absorbing surface area and disperse absorbed heat very rapidly, whereas more dissected leaves keep the leaves cooler and thus enhance photosynthesis (Hegazy and El Amry, 1998; Smith, 1978).

Internal leaf anatomy

Accompanying leaf size and shape variation is variation in the internal leaf anatomy. The internal leaf anatomy is complex and is one of the several factors that help determine photosynthetic capability of plants (Smith, 1997, Kalenhoff, 2012). The internal tissue of the leaf comprises epidermis, mesophyll and the vascular tissue. A transverse section passing through the midrib region of a *Begonia* leaf presents the epidermal and mesophyll features of the internal leaf anatomy in Figure 3.1.

Epidermal variation

Tremendous variation is exhibited by the epidermises of leaves. Epidermal cells serve as a boundary layer between the leaf's internal atmosphere and the external environment and are responsible for providing light to the mesophyll for carrying out the process of

photosynthesis. Epidermal cells have evolved over time to serve many purposes. They have modified to retain water, control transpiration and CO_2 uptake and discourage predation by insects as well as fungi and bacteria (Bone et al., 1985). These cells also protect mesophyll cells against harmful waves by absorbing UV radiation (Smith et al., 1997). Epidermal cell shape can lead to them functioning like lenses. Many shade or understory plants possess convex shaped epidermal cells with a higher water content and are geared for focusing light as it passes into the leaf (Vogelmann, 1993; Bone et al., 1985). A few studies have also reported their role in preventing the formation of a water layer on the leaf surface (Vogelmann et al., 1996).

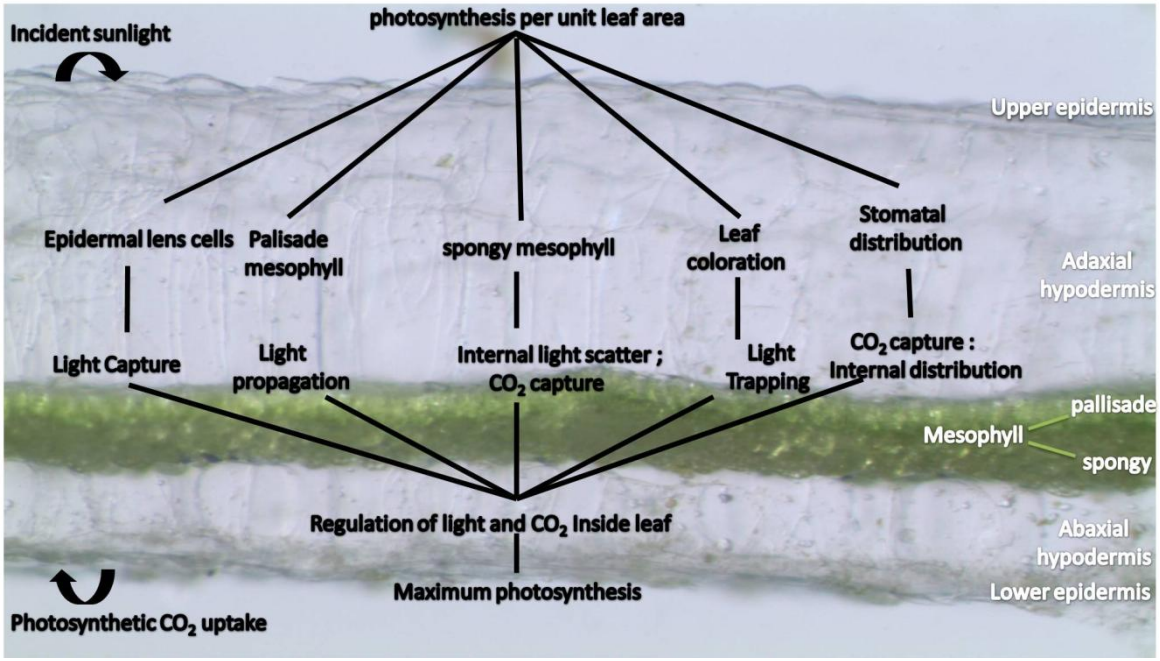

Figure 3.1: Transverse section of *B. conchifolia* leaf and a schematic diagram of leaf structural components involved in regulation of CO_2 and light inside leaf to maximise photosynthesis per unit leaf area. Adapted from Smith et al. (1997)

Some epidermal cells develop into trichomes that vary to a great extent in type, size and the density. The presence of trichomes on the leaf surface alters the amount of light absorbed by the leaf, resulting in reduced leaf temperature and potentially reducing the transpiration rate

by trapping humid air next to the leaf. The lower levels of light absorbed by the leaf surface improve the overall net photosynthetic rate (Ehlinger and werk 1986; Smith, 1978). Another kind of trichome known as glandular trichomes can play a role in dispersion of extreme radiation in desert plants. These trichomes secrete exudate that forms a thick layer on the leaf surface and helps in reflecting the light and reducing leaf temperature (Tattini and Gucci, 1999).

Stomatal variation

Stomatal apertures present on the epidermis are key features responsible for gas exchange between the leaf and atmosphere (Schroeder et al., 2001). The number, distribution, and size of stomata are species specific and environmentally modifiable. The underlying theme behind the evolution of stomatal aperture attributes is to facilitate gaseous exchange between leaf and atmosphere. Species found in sunny habitats tend to have high stomatal density, whereas species found in shady habitats have low stomatal density. Variation has also been documented for stomatal sizes. The size of stomata for plants found in sunny habitats is small whereas plants in shady habitats have large stomatal apertures (Larcher, 2003).

Mesophyll variation

The internal distribution of sunlight for regulating photosynthesis is carried out by mesophyll (Vogelmann, 1993; Vogelmann et al., 1996a). Sun leaves are characterised by columnar pallisade cells that are better able to focus light deeper into the mesophyll. To increase the absorption of light within the mesophyll, shade plants are characterised by cells that are spherical in the spongy mesophyll which along with a large fraction of air spaces in the interior of the leaf generates large quantities of scattered light. Vogelmann (1993) illustrated that the scattering of light within the mesophyll was able to generate photon fluence levels

three to four times greater than sunlight incident on the leaf surface, enhancing the absorption of weakly absorbed wavelengths in particular.

Vascular trait variation

Vascularisation pattern refers to leaf conducting tissue which offers a network for water, nutrients, and carbon supply for nearly all plants. Several studies have reported that venation patterning and the vascular system of a plant could play an important role in providing a transport system capable of matching the net assimilation rates of a leaf (Hofstra and Nelson, 1969). Broadribb and colleagues (2007) also confirmed the potential role of leaf vasculature in gaseous exchange efficiency. They measured the density of the veins and thickness of the mesophyll in 43 species across the breadth of plant diversity from mosses to flowering plants and determined strong association of these traits with net assimilation rate.

3.2 Aims of the project

This study investigated the hypothesis that species will differ significantly in ecophysiological and morphological traits if section Gireoudia speciated through habitat adaptation to specific environments.

3.3 Results

Described below are the general plant morphological, leaf size and shape attributes, micromorphological, and resource use strategy traits. The traits scored in each category are described in detail with reference to the codes used for them in the study. Each species was represented by two plants of the same accession, therefore clones. For micromorphological traits an average measure was calculated from three fields of views for each trait.

3.3.1: General plant morphology

Variation in general plant morphological traits in *Begonia* section Gireoudia has already been documented in the monograph on Section Gireoudia and in specific species descriptions (Burt-Utley, 1985 and references within Burt-Utley, 2012). The morphological traits that were scored in this category included rhizome architecture and leaf production. A total of six categorical and seven quantitative traits were measured to assess differences in plant architecture in *Begonia* section Gireoudia. The summary of morphological traits is presented in Table 3.1. Below is a detailed description of the traits measured in this category.

a. Habit

Species in section Gireoudia can be grouped based on whether the plants are suffrutrescent with erect stems (having a woody base that does not die down each year) or are rhizomatous. Only three of twenty one American species in this study are suffrutrescent herbs: *B. corredorana, B. multinervia, and B. involucrata* (coded as 1). The remaining eighteen species are caulescent (having a well-developed aboveground stem) or acaulescent (having no stem) rhizomatous herbs (coded as 0).

b. Anthocyanin production

Anthocyanin pigmentation was scored on petioles and leaves (Figure 3.2). The blades in *Begonia* section Gireoudia are medium to dark green on their adaxial surface, occasionally becoming red marginally, and are generally lighter beneath, but in *B. multinervia* the lower surface is often deeply pigmented with anthocyanins and appears deep maroon. Some leaves such as *B. plebeja, B. mazae, B. hydrocotilifolia,* and *B. lindiliyana* have deep red blotches throughout the lamina (coded as 1 against non-blotched leaves that are coded as 0). Some of the leaf blades have anthocyanins in the leaf margins. These species include *B. thiemie, B. squarrosa* and *B. cardiocarpa* (these species are coded as 1 against species that lack red

margins and are coded as 0; the rest of the species in the sample). Anthocyanin pigmentation also extends to petioles and petiole attachment points. The pigmentation at petiole attachment point is referred to as red eye in this study. Among the species in section Gireoudia, the red eye is characteristic of the *B. plebeja* and *B. lindileyana* leaf. The strength of red eye pigmentation was also measured in this study. Red eye was evaluated based on three categories. The strength varied from no red eye (coded as 0, most of the Gireoudia fit in this category) to pink (coded as 1, *B. lindleyana*) to red (coded as 2, *B. plebeja*).

Petiole colour also varies in strength in the section. The strength of petiole colour was coded as 0 for no red colouration, 1 for weak red colouration, 2 for medium red colouration, 3 for strong red colouration, and 4 for multi-coloured (white with red sections). For example, *B. plebeja* is characterised by green petioles whereas *B. conchifolia* has strong red petioles. The trait petiole colouration was also recorded by Burt-Utley in her revision of Central American species of *Begonia* section Gireoudia (1985). The comparison of both the data sets revealed some incongruities with the field observations suggesting that anthocyanin pigmentation traits are strongly under the effect of environment or possibly intraspecific variation for this trait as is common for anthocyanin traits (Table 3.3).

c. Rhizome architecture

Within rhizomatous species two different growth forms are prevalent, depending upon rhizome orientation which may be characterised as erect to ascending in one group and repent in the other (Burt-Utley, 1985). In both groups the older portions of the rhizome gradually undergo senescing and die (Burt-Utley, 1985). Of the 21 species studied, only two have generally ascending rhizomes, *B. cardiocarpa* and *B. sericoneura* (Burt-Utley, 1985).

In most of the species that have characteristically repent rhizomes, the rhizomes may either be unbranched or only sparingly branched, but some species have rhizomes that branch freely, forming dense mats. Within these species the rhizomes are often thick and succulent that may function as moisture reservoirs enabling the species to endure prolonged dry seasons in exposed habitats. Rhizome growth in *Begonia* section Gireoudia is either periodic or continuous (Burt-Utley, 1985).

In this survey, rhizome architecture was scored by scoring the total number of rhizomes, rhizome length and rhizome width. The total number of rhizomes produced on a mature plant varied from three (*B.nelumbifolia*) to eleven (*B. carolinefolia*). On average the length of rhizomes varied from 9.10 mm (*B. mazae*) to 186.75 mm (*B. sericoneura*). The length of rhizomes values were well in the range of what has been observed under natural field conditions by Burt-Utley (1985). The comparison has been drawn in Table 3.3. The average width of rhizomes varied from 0.62 mm (*B. mazae*) to 37.64 mm (*B. heracleifolia*).

d. Leaf morphology and production

Section Gireoudia presents remarkable diversity for traits related to leaf size and shape as well as production rate. Most of the species in this section produce leaves that are persistent. However, some rhizomatous species that possess succulent rhizomes are adapted to seasonally dry habitats and some populations are deciduous (for example, *B. plebeja* and *B. heracleifolia*). However, in the greenhouse conditions the accession used here were not reliably deciduous.

Most of the species in *Begonia* section Gireoudia produce simple leaves (with the exception of *B. thiemie*, and *B. carolinefolia*). Another level at which species could be categorized is at the level of peltate or non peltate leaf production. The difference between both the kinds of

leaves is at the petiole attachment point at the leaf. Species that produce peltate leaves have their petiole attached at the centre of the leaf whereas a non peltate leaf has its petiole attached at the end of the leaf. *B. peltata* and *B. conchifolia* are the only species of this sample that produce peltate leaves.

Great differences in **leaf production** are observed among the mature specimens of in section Gireoudia in our collection. Leaf number varied from a minimum of two (*B. heracleifolia*) to two hundred and forty five leaves (*B. hydrocotylifolia*).

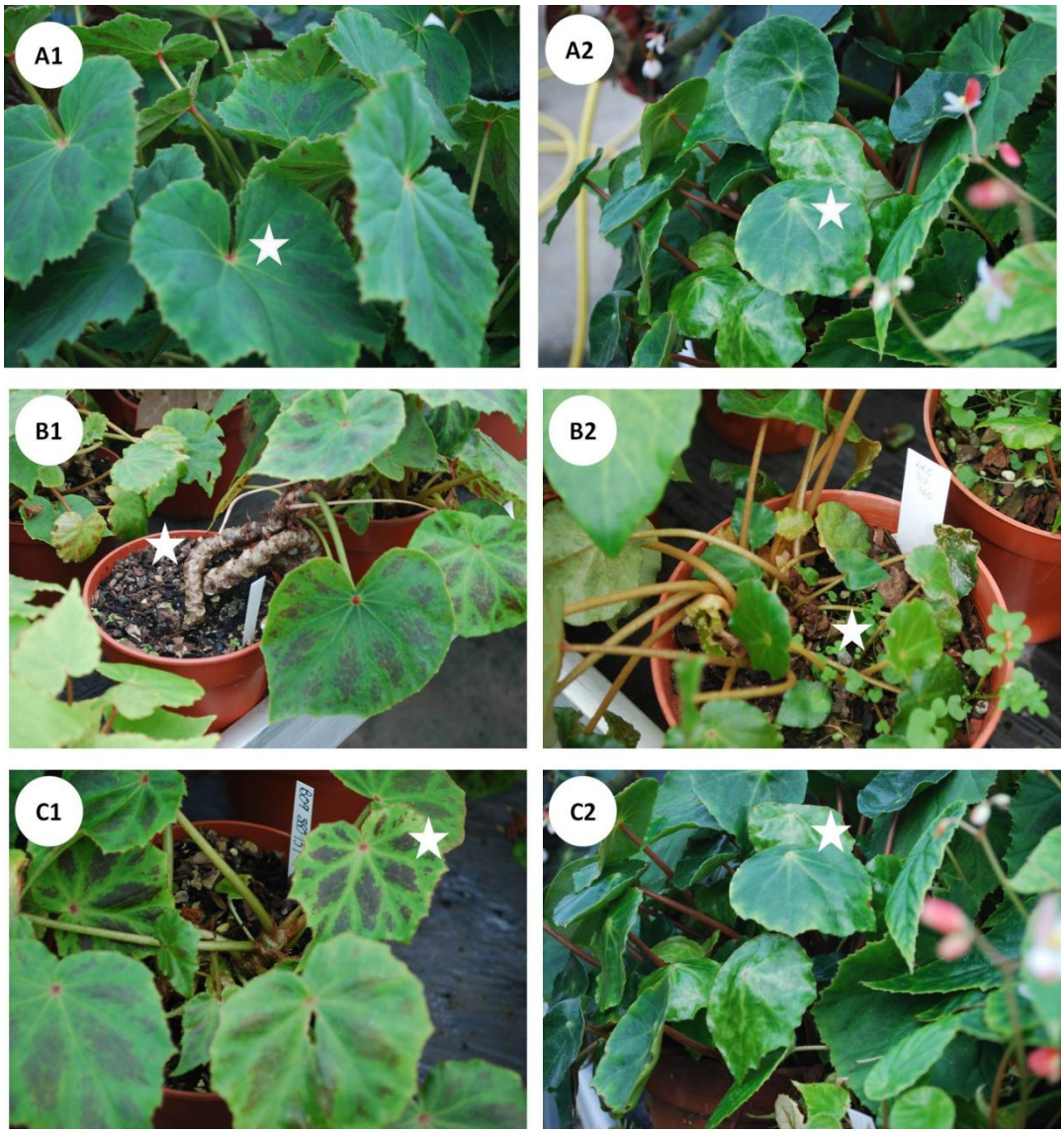

Figure 3.2: The presence and absence of red petiole base (A1 & A2), presence and absence of blotches (B1 & B2), and presence and absence of red margins (C1 & C2) in *B. plebeja*(1) and *B. conchifolia* (2). These species are growing in the green house conditions in Royal Botanic garden Edinburgh.

Table 3.1: General plant morphological traits in 21 *Begonia* species in section Gireoudia. p. The traits measured in this category included presence/absence of blotches (B), red eye (RE), red margin (RM), petiole colour (PC), habit type (H1), simple versus compound leaf (SC), total number of rhizomes (TOR), average length of rhizomes (LOR), average width of rhizomes (WOR), total number of scars (TNS), total number of leaves (TNL), and total number of scars and leaves produced (TNSL). The length and width of rhizomes were measured in mm.

Species	B	RE	RM	PC	SC	TNR	LOR	WOR	TNS	TNL	TNSL
B. mazae	1	0	0	0	0	10	9.1	0.6	4	10	14
B. lindliyana	1	1	0	2	0	6	75.6	3.4	71	14	85
B. purianata	0	0	0	0	0	6	66.5	23.1	34	43	77
B. peltata	0	0	0	1	0	9	111.8	13.1	43	34	77
B. carriae	0	0	0	0	0	4	80.0	17.8	53	7	60
B. conchifolia	0	0	0	3	0	9	46.2	7.4	48	84	132
B. plebeja	1	2	0	0	0	10	125.0	16.9	137	40	177
B.kellermani	0	0	0	0	0	10	32.5	4.6	49	33	82
B. sericoneura	0	0	0	0	0	4	186.7	12.2	72	21	93
B. corredorana	0	0	0	0	0	5	174.0	6.08	31	7	38
B. squarrosa	0	0	1	4	0	5	63.0	10.0	25	43	68
B. heracleifolia	0	0	0	3	1	6	79.6	37.6	49	2	51
B. multinervia	0	0	0	3	0	6	182.6	7.5	11	39	50
B. hydrocotylifolia	1	0	0	0	0	9	65.3	7.4	82	163	245
B. nelumbiifolia	0	0	0	1	0	3	80.0	25.6	35	12	47
B. carolineifolia	0	0	0	0	1	11	117.7	26.5	102	39	141
B. stigmosa	0	0	0	0	0	5	71.6	16.2	20	82	102
B. thiemie	0	0	1	3	1	7	62.1	10.6	42	34	76
B. multistaminae	0	0	0	0	0	2	176	17.	28	15	43
B. involucrata	0	0	0	0	0	7	134.5	6.4	50	11	61
B. cardiocarpa	0	0	1	0	0	6	146.5	6.6	82	49	131

3.3.2: Leaf size and shape attributes

Leaf shape and size is highly variable within *Begonia* section Gireoudia. Leaf shape ranges from suborbicular through broadly elliptic or oblong to ovate or obovate, to simple or dissected or compound (Burt-Utley, 1985). Leaf size differences are also observed among leaves of different species, within a species population and even on a single plant. Figure 3.3 describes the variability for leaf shape and size within the section.

Leaf size and shape traits for my sample were scored with the help of Lamina software (Bylesjo et al., 2008). A total of eleven quantitative traits were measured for two leaves per species (Table 3.2). Given below is the list of traits measured and the range of values observed for each trait

a. Leaf area

Species in section Gireoudia exhibit huge variation in the surface area exposed to the light for carrying out the process of photosynthesis. Quantitative data indicated that *B. conchifolia* leaf has the smallest leaf surface area (average 930.3 mm^2), while on the other hand *B. nelumbifolia* produces the largest leaf surface area (average 20561.2 mm^2).

c. Leaf perimeter & squared perimeter / Area

Simple descriptors of shape such as Leaf dissection Index LDI = perimeter / [√area] was used to investigate both variations in leaf margin (e.g. dentate margins). Among the species in section Gireoudia, the perimeter to area ratio is smallest for *B. hydrocotilifolia* (11.2) and highest for *B. thiemie* (102.39). The highly dissected leaves of *B. thiemie* could be associated with high transpiration rates owing to their high perimeter-area ratios (Canny, 1990).

d. Circularity

Circularity (Area/perimeter^2) indicates that among the species in section Gireoudia, *B. thiemie* is the least circular species (also confirmed by leaf dissection index) while *B. hydrocotilifolia* has the highest circularity index.

e. Length & width of the leaf

The length of the leaf values for the species in the section ranged from 53.49 mm (*B. hydrocotilifolia*) to 201.82 mm (*B. nelumbifolia*). Leaf width varied from 31.49 mm (*B. conchifolia*) to 178.68 mm (*B. heracleifolia*).

f. Horizontal and vertical symmetry

Horizontal (left-right) and vertical symmetry (dorsiventral) were also observed among the species in the section Gireoudia. A leaf is symmetrical only if it is congruent on both sides. When the horizontal leaf symmetry is taken into account *B. nelumbiifolia* has the least horizontally symmetrical leaves whereas *B. multinervia* leaf is nearly horizontally symmetrical. *B. thiemie* leaf is the least vertically symmetrical with a value of 0.62 and *B. involucrata* the most vertically symmetrical with a value of 1.22.

g. Number of serrations

To investigate variation in leaf toothiness at the margins, the numbers of serrations were measured using margin analysis in LAMINA. According to the values observed *B. mazae* leaf is the least serrated with an average of 11.5 serrations while *B. carolinefolia* is the most serrated with an average of 65.5 indents.

h. Indent depth & width

Species in the section also varied with respect to the depth and width of serrations. Serrations were less deep in case of *B. conchifolia* (0.73 mm) and very deep in case of *B. heracleifolia* (3.27 mm). The width of the serrations varied from an average of 7.69 mm (*B. conchifolia*) to 14.03 mm (*B. nelumbiifolia*).

Comparison of leaf size attributes under field and green house conditions

The leaf size data was compared to the data obtained by Burt-Utley (1985) in her revision of Central American species of *Begonia* section Gireoudia. The values in the green house obtained for the leaf blade size were much in the range of those obtained by Burt-Utley (1985) on field plants (Table 3.3).

Figure 3.3: Leaf size and shape variation in Central American *Begonia* species of section Gireoudia. The images were generated by the lamina software. The figure presents cropped images of the species of *Begonia* section Gireoudia. *B. multinervia* (1), *B. thiemie* (2), *B. heracleifolia* (3), *B. hydrocotilifolia* (4), *B. imperialis* (5), *B. mazae* (6), *B. lindliyana* (7), *B. kellermani* (8) *B. multistaminae* (9), *B. nelumbifolia* (10), *B. peltata* (11), *B. plebeja* (12), *B. purianata* (13), *B. carriae* (14), *B. cardiocarpa* (15), *B. squarrosa* (16), *Stigmosa* (17), *B. carolinefolia* (18), *B. sericoneura* (19), *B. conchifolia* (20), *B. corredorana* (21). These species are growing in the glass house conditions in Royal Botanic garden Edinburgh.

Table 3.2: Summary of leaf size and shape traits in *Begonia* section Gireoudia. The number of replicates used is 2. The traits measured in this category included area of leaf (A), leaf perimeter (P), perimeter/Area (P/Area), circularity (c) , lamina leaf width (w), lamina leaf length (l), Horizontal symmetry (H.symm), vertical symmetry (v.symm), number of indents (Nind), indent width mean (Iwm) and indent depth mean (Idm). The traits measured were expressed in mm.

List of species	A	P	p/Area	C	w	l	H.symm	v.symm	Nind	Iwm	Idm
Begonia mazae	4595.3	235.97	12.17	87.42	69.55	93.9	0.93	0.92	11.50	10.3	1.34
B. lindliyana	10918.51	366.79	12.98	89.55	110.1	132.41	1.02	1.14	23.25	12.57	1.51
B. purianata	10430.36	352.71	12.16	86.33	108.53	137.89	0.79	1	20.33	12.52	1.18
B. peltata	12875.88	365.31	11.24	93.3	112.5	143.02	0.8	0.98	23.33	11.68	0.88
B. carriae	7462.35	363.81	17.92	83.06	97.09	117.3	0.84	1.01	28.50	9.86	1.52
B. conchifolia	930.32	100	11.55	88.82	31.49	39.15	0.77	0.96	6.33	7.69	0.73
B. plebeja	7954.46	337.11	16.51	80.11	92.54	117.38	0.77	1.02	27.83	11.03	1.76
B. kellermani	5714.27	256.4	11.52	88.05	74.32	107.46	0.84	1.01	16.67	11.06	0.83
B. sericoneura	11694.04	396.51	13.85	89.31	118.43	137.33	1.02	1.19	27.4	11.82	1.53
B. corredorana	7370.88	313.52	13.37	79.94	89.39	127.03	0.69	1	23.0	9.57	1.04
B. squarrosa	5656.83	277.71	13.7	85.51	72.87	110.47	0.7	0.85	18.67	11.85	1.19
B. heracleifolia	20300.19	831.78	49.77	70.94	178.68	125.68	0.94	1.04	44.33	13.85	3.27
B. multinervia	15203.95	436.77	12.56	97.54	128.63	152.76	1.16	1.08	22.5	12.33	1.29
B. hydrocotylifolia	2494.82	167.15	11.2	98.68	59.98	53.49	1.02	1.14	7.33	9.21	0.93
B. nelumbiifolia	20561.28	508.65	13.26	81.71	147.02	201.82	0.52	1.06	38.0	14.03	1.37
B. carolineifolia	13640.73	1044.19	88.62	70.5	139.97	169.62	1.11	0.69	65.5	12.49	2.58
B. stigmosa	10218.47	359.79	12.76	94.01	115.21	119.57	0.93	1.1	23.67	13.22	1.4
B. thiemie	11804.65	1081.4	102.39	56.96	176.89	148.4	1.02	0.62	78.5	10.61	1.66
B. multistaminae	9748.3	353.34	12.91	94.15	111.34	117.73	0.93	1.15	23.0	13.43	1.45
B. involucrata	8005.43	327.62	13.41	84.19	91.28	128.78	1.08	1.22	21.0	10.42	1.39
B. cardiocarpa	12510.48	448.84	16.1	81.98	115.12	163.66	0.55	0.76	27.0	12.75	1.81

Table 3.3: Comparison of leaf blade size, petiole colouration trait under controlled environment (MSA) and field conditions (Burt-Utley).

	leaf blade size (BU)	leaf blade size (MSA)	petiole colouration (BU)	petiole colouration MSA
B. conchifolia	2-9(14) x 1-8(10)	3.6 x 2.8	maroon to light green	red
B. plebeja	6-18(23) x 4-13(18)	13.5 x 9.4	maroon to light green	green
B. sericoneura	(4)7-14(22) x (4)6-12(16)	14.3 x 12	light green with red pigments	green
B. corredorana	(13.5) 16-28.2 x (7.4)9-15.5(19)	16.1 x 12.3	light green with red pigments	green
B. heracleifolia	4-26 x 3-24	20 x 31	NA	NA
B. multinervia	7-25(29.1) x 3-15(18.5)	12.1 x 8.1	maroon to light green	red
B. nelumbiifolia	15-40 x 11-32	25.2 x 18	NA	NA
B. thiemie	21-54 x 20-39	13 x 20	NA	NA
B. involucrata	(8.7)11.2-27.5(33) x (5.6)8.8-17.2(22.3)	14 x 11.2	light green	green
B. cardiocarpa	(5.5) 7-21(30) x (3.2)5-13(21.5)	17 x 11.5	light green	green

3.3.3: Leaf micromorphological traits

Morphological characters of the leaf and inflorescence have been used to distinguish the species among *Begonia* section Gireoudia (Burt-Utley, 1985). However additional characters related to leaf micromorphology were scored to further dissect variation between species. The present investigation is also the first detailed qualitative and quantitative study of anatomical features for the species of *Begonia* section Gireoudia. Traits such as number, distribution, size of stomata, as well as number, distribution and size of trichomes were scored. In all cases the number of replicates was two. Three samples per leaf per plant were recorded and averaged.

3.3.3.1: Leaf Anatomy

Table 3.5 presents leaf anatomical variation for species within section Gireoudia. Given below is the list of anatomical traits measured and the range of values observed (Figure 3.4).

a. Epidermal cell type

Epidermal cells can either appear flat or peaky. The survey of leaf anatomical traits confirmed the presence of both flat and peaky epidermal cells in *Begonia* section Gireoudia. The shape of the epidermal cell was coded as 0 for flat and 1 for peaky epidermal cell types. Table 3.4 lists the type of abaxial and adaxial epidermal cell for the species in this section.

Table 3.4: Epidermal cell types

Specie	Adaxial epidermal cell type	Abaxial epidermal cell type
B. mazae	peak	flat
B. lindiliyana	peak	flat
B. pruniata	flat	flat
B. peltata	flat	flat
B. carriae	peak	flat
B. conchifolia	flat	flat
B. plebeja	peak	peak
B.kellermani	flat	flat
B. sericoneura	peak	peak
B. corredorana	flat	flat
B. squarrosa	peak	peak
B. heracleifolia	peak	peak
B. multinervia	flat	flat
B. hydrocotylifolia	flat	flat
B. nelumbiifolia	flat	flat
B. carolineifolia	flat	flat
B. stigmosa	flat	flat
B. thiemie	flat	flat
B. multistaminae	flat	flat
B. involucrata	flat	flat
B. cardiocarpa	flat	flat

b. Lamina depth at vein

The lamina depth at the principal vein of the leaf also varied between the species in the section, from an average of 0.24 mm (*B. thiemie)* to 0.66 mm (*B. conchifolia*).

c. Depth of adaxial and abaxial epidermal cells

The depth of adaxial and abaxial epidermal cells varied to a little extent among the species in the section. At the adaxial side, the leaf epidermal cell depth ranged from an average of 0.02 mm (*B. thiemie*) to 0.07 mm (*B. plebeja*). In comparison to the adaxial side, leaf epidermal cells depth at the abaxial side did not much and quite a few species had an average epidermal cell depth of 0.03 mm including *B. plebeja* and *B. heracleifolia*.

d. Shape of adaxial and abaxial hypoderm cells

Abaxial and adaxial hypoderm cells also varied in their shape. Four different types of cells could be categorised on shape including elliptical polyhedral cuboidal (1), polyhedral cuboidal (2), polyhedral spheriodal (3), and polyhedral (4). See Table 3.5 for details.

e. Depth at adaxial and abaxial hypodermis

A layer of thick walled, non-photosynthetic cells immediately below the epidermis is called the hypodermis. Variation observed for quantitative hypodermal attributes among the species. The total depth of the adaxial hypodermis ranged from 0.05 mm (*B. involucrata*) to 0.39 mm (*B. hydrocotilifolia*). On the abaxial side of the leaf the depth of the hypodermis ranged from an average of 0.03 mm (*B. thiemie* and *B. carolinefolia*) to 0.2 mm (*B. conchifolia*).

f. Width of adaxial and abaxial hypoderm cells

The cells in the adaxial and abaxial hypodermis varied in their width. At the adaxial side of the leaf, cell width varied from an average of 0.04 mm (*B. cardiocarpa*) to 0.11 mm (*B.*

hydrocotilifolia). At the abaxial side, hypodermal cell width varied from an average of 0.05 mm (*B. carolinefolia*) to 0.1 mm (*B. plebeja*).

g. Mesophyll attributes

Mesophyll is structured (i.e., distinct pallisade and spongy mesophyll are present) in most of the species except *B. involucrata*. Variation is observed in pallisade and spongy mesophyll depth among the species of the section. Mesophyll depth varied from an average of 0.07 mm (*B. carriae*) to 0.15 mm (*B. hydrocotilifolia*). On the other hand depth of pallisade mesophyll ranged from an average of 0.02 mm (*B. multistaminae*) to 0.1 mm (*B. squarrosa*). Depth of spongy mesophyll ranged from an average of 0.02 mm (*B. carriae*) to 0.12 mm (*B. carolinefolia*).

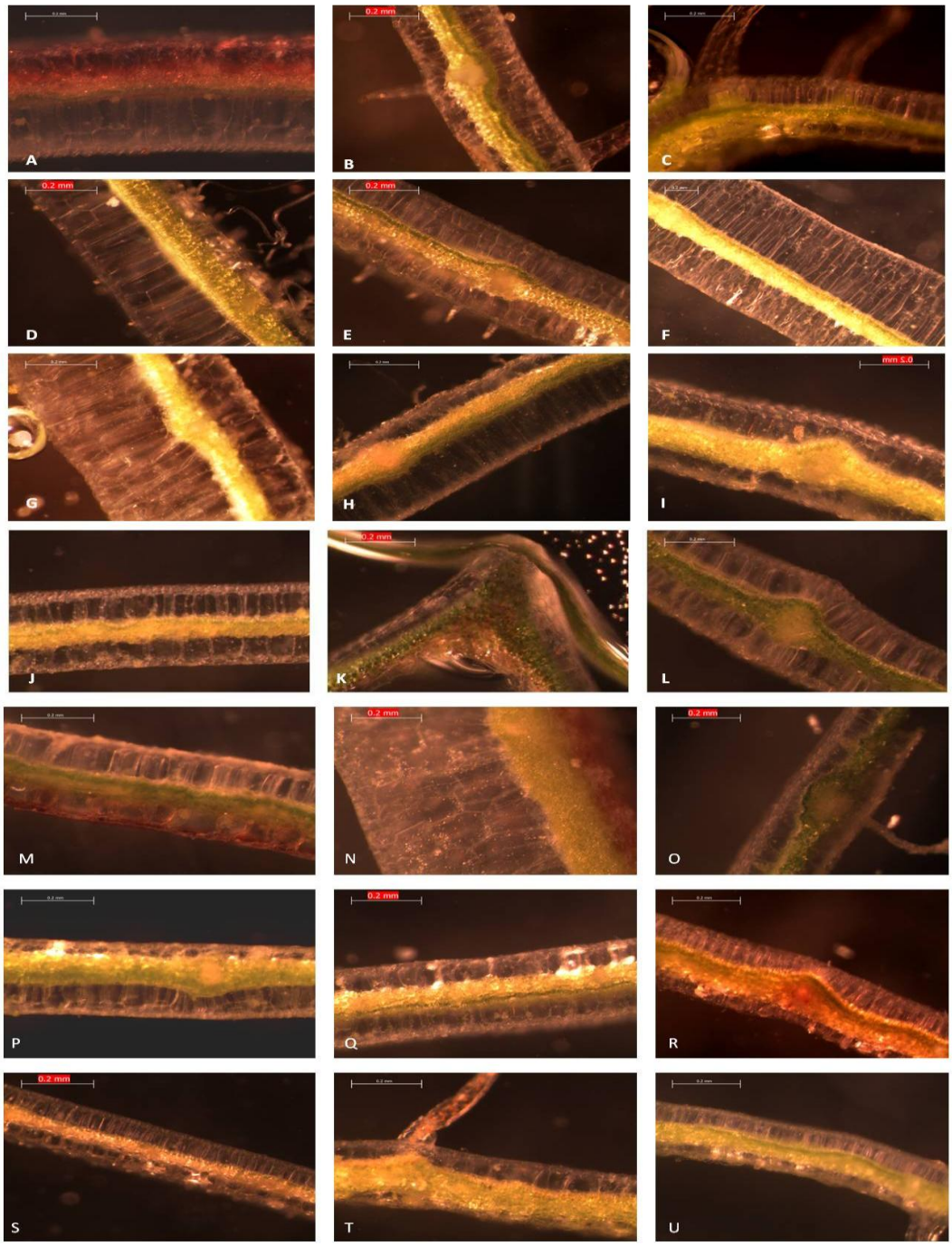

Figure 3.4: Leaf transverse sections for : *B. mazae* (A), *B. lindiliyana* (B), *B. carrieae* (C), *B. puriniata* (D), *B. peltata* (E), *B. conchifolia* (F), *B. plebeja* (G), *B. kellermani* (H), *B. sericoneura* (I), *B. squarrosa* (J), *B. imperialis* (K), *B. heracleifolia* (L), *B. multinervia* (M), *B. hydrocotylifolia* (N), *B. nelumbiifolia* (O), *B. carilinefolia* (P), *B. stigmosa* (Q), *B. thiemie* (R), *B. multistaminae* (S), *B. involucrata* (T), *B. cardiocarpa* (U).

Table 3.5: Summary of leaf anatomical traits in *Begonia* section Gireoudia. The traits measured in this category included depth at lamina (wl), depth at vein (wv), depth at adaxial and abaxial epidermis (wde & wbe), depth at adaxial and abaxial hypodermis (wdh & wbh), width at adaxial and abaxial cell (wdc & wbc), number of adaxial and abaxial hypoderm cell (Ndhc & Nbhc), type of adaxial and abaxial hypoderm cell (Tdhc & Tbhc), depth of mesophyll (wm), mesophyll structured (ms), and depth of pallisade and spongy mesophyll (dpm & dsm). The traits were measured in mm^2.

Species	Wl	wv	wde	wdh	wdc	Ndhc	Tdhc	wbe	wbh	wbc	Nbh	Tbh	wm	ms	dpm	dsm
B. mazae	0.42	0.43	0.03	0.17	0.08	2	4	0.02	0.09	0.08	1	2	0.11	1	0.03	0.07
B. lindiliyana	0.26	0.26	0.02	0.06	0.06	1	4	0.02	0.05	0.05	1	4	0.09	1	0.04	0.05
B. pruniata	0.38	0.39	0.06	0.13	0.07	2	4	0.02	0.08	0.07	2	4	0.11	1	0.03	0.07
B. peltata	0.45	0.44	0.03	0.15	0.08	2	4	0.02	0.04	0.06	1	3	0.11	1	0.06	0.53
B. carriae	0.25	0.26	0.03	0.06	0.05	1	2	0.03	0.05	0.05	1.5	3	0.07	1	0.03	0.04
B. conchifolia	0.64	0.66	0.04	0.23	0.08	2.3	4	0.03	0.2	0.08	1.6	4	0.14	1	0.04	0.1
B. plebeja	0.54	0.53	0.07	0.28	0.1	1.6	4	0.03	0.09	0.1	1	3	0.1	1	0.04	0.06
B.kellermani	0.30	0.28	0.02	0.11	0.06	1	1	0.02	0.04	0.05	1	3	0.09	1	0.03	0.05
B. sericoneura	0.33	0.33	0.03	0.09	0.06	2	4	0.03	0.05	0.06	1	1	0.10	1	0.03	0.06
B. corredorana	0.24	0.29	0.02	0.05	0.05	1	1	0.03	0.04	0.06	1.5	3	0.09	1	0.03	0.06
B. squarrosa	0.28	0.28	0.03	0.08	0.07	1	2	0.02	0.07	0.09	1	3	0.09	1	0.16	0.05
B. heracleifolia	0.29	0.32	0.03	0.09	0.06	1.5	2	0.03	0.07	0.05	1	2	0.08	1	0.03	0.05
B. multinervia	0.32	0.34	0.03	0.09	0.06	1	2	0.03	0.07	0.08	1	3	0.10	1	0.04	0.06
B. hydrocotilifolia	0.64	0.65	0.02	0.39	0.11	3	4	0.01	0.12	0.08	1	4	0.15	1	0.05	0.10
B. nelumbiifolia	0.26	0.26	0.03	0.07	0.07	1	2	0.02	0.06	0.06	1	3	0.09	1	0.04	0.05
B. carolineifolia	0.30	0.31	0.02	0.09	0.06	1	2	0.02	0.03	0.05	1	3	0.13	1	0.10	0.12
B. stigmosa	0.31	0.29	0.03	0.06	0.07	1	2	0.02	0.08	0.07	1	2	0.11	1	0.03	0.07
B. thiemie	0.24	0.24	0.02	0.06	0.06	1	2	0.02	0.03	0.06	1	3	0.12	1	0.07	0.06
B. multistaminae	0.31	0.52	0.03	0.1	0.05	1	2	0.02	0.05	0.04	1	3	0.09	1	0.02	0.06
B. involucrata	0.23	0.28	0.02	0.05	0.04	1	2	0.01	0.03	0.03	2	3	0.11	2	NA	NA
B. cardiocarpa	0.18	0.23	0.02	0.04	0.01	1	2	0.01	0.03	0.03	2	3	0.07	1	0.02	0.04

3.3.3.2: Stomatal survey of *Begonia*

A stomatal survey was carried out to (a) score qualitative and quantitative stomatal trait variation among the species in *Begonia* section Gireoudia and (b) understand the developmental process and mechanism of singly occurring stomata and stomatal clusters in *Begonia* section Gireoudia.

I. Qualitative and quantitative stomatal trait variation

Microscopic investigations revealed that *Begonias* in section Gireoudia are hypostomatous i.e; stomata occur mostly on the abaxial side of the leaf surface. Both solitary and clustered stomata are found on the *Begonia* leaves of most species (Table 3.6). The stomata in *Begonia* section Gireoudia are anisocytic i.e., the stoma are surrounded by three cells, one of which is smaller (Metcalfe and Chalk, 1950; Pant and Kidwai, 1967).

Light microscopy was used to score stomatal cluster density, the length and width of clusters and the number of stomata in each cluster. Stomatal density varies among the species in section Gireoudia. The number of stomatal clusters per mm^2 of leaf ranges from an average of 90 (*B. squarrosa*) to 375.8 (*B. thiemie*). The smallest distance between clusters is found for *B. involucrata* (0.04 mm), *B. heracleifolia* has the largest distance between clusters (0.46 mm). Substomatal cluster width varies from 0.05 mm (*B. hydrocotilifolia*) to 0.28 mm (*B. heracleifolia*). The substomatal cluster length varies from 0.02 mm (*B. involucrata*) to 0.23 mm (*B. heracleifolia*). On average the number of stomata per stomatal cluster falls in the range of one to four in *Begonia* section Gireoudia **(**Table 3.7).

Table 3.6: Summary of type of stomata and trichomes present on *Begonia* leaves. The number of replicates used is two. The presence of stomata are categorised into single, clustered and both types present whereas the trichomes are categorised as whiplash, glandular and villi/scale.

Specie	Type of stomata		Vestiture		
	Single	Cluster	whiplash	glandular	Villi / scale
B. mazae		✓	✓	✓	
B. lindiliyana			✓	✓	
B. purianata	✓	✓	✓	✓	
B. peltata	✓	✓	✓	✓	
B. carriae	✓	✓			✓
B. conchifolia	✓	✓	✓	✓	
B. plebeja		✓	✓	✓	
B.kellermani	✓	✓	✓	✓	
B. sericoneura	✓	✓	✓	✓	
B. corredorana					
B. squarrosa	✓	✓	✓	✓	
B. heracleifolia	✓	✓			✓
B. multinervia		✓	✓	✓	
B.hydrocotilifolia	✓		✓	✓	
B. nelumbifolia	✓	✓	✓	✓	
B. carolinefolia	✓		✓	✓	
B. stigmosa	✓	✓	✓	✓	
B. thiemie	✓				✓
B. multistaminae	✓	✓	✓	✓	
B. involucrata	✓				✓
B. cardiocarpa	✓	✓			✓

II. Developmental process and mechanism of singly occurring stomata

This section describes the development of stomatal clusters through time to investigate the formation and pattern of stomatal clusters. *B. sericoneura* was chosen to score the development of solitary and clustered stomata in section Gireoudia. *B. sericoneura* possesses both single and clustered stomata on its leaves and was a good choice to scan the developmental processes using dental resin impression methods as well as scanning electron microscopy.

Stomatal development in *B. sericoneura*

An SEM scan of a juvenile leaf of *B. sericoneura* suggested that stomatal development starts after the trichomes have reached maturity (Figure 3.5A). Both solitary and clustered stomata are present on *B. sericoneura* leaves. At the onset of stomatal cluster development a single cell differentiates into a precursor cell (Figure 3.5B). The precursor cell undergoes divisions and produces daughter cells, one of which differentiates into a meristemoid mother cell (Figure 3.5C). The meristimoid mother cells then undergo a further division, producing meristimoid cells that are adjacent to each other (Figure 3.5C). Meristimoid cells could be easily recognised by their small size and triangular shape. The meristimoid cells then undergo a further series of asymmetrical divisions to form a large cell and a small triangular cell at the corner. The larger cell of the meristimoid cell differentiates into the first subsidiary cell, while the small meristimoid cell remains meristematic. The small meristimoid cell then enlarges to become a round guard mother cell (Figure 3.5D). The round guard mother cell then undergoes a final symmetrical division to form two guard cells of equal size (Figure 3.5E). After this crucial step pore formation takes place. Pore formation finally results by the separation of the anticlinal walls (Figure 3.5E). Satellite meristimoids have also been

observed in some of the *Begonia* species such as *B. imperialis*. They originate from the meristimoid mother cells that are located next to a pre- stoma.

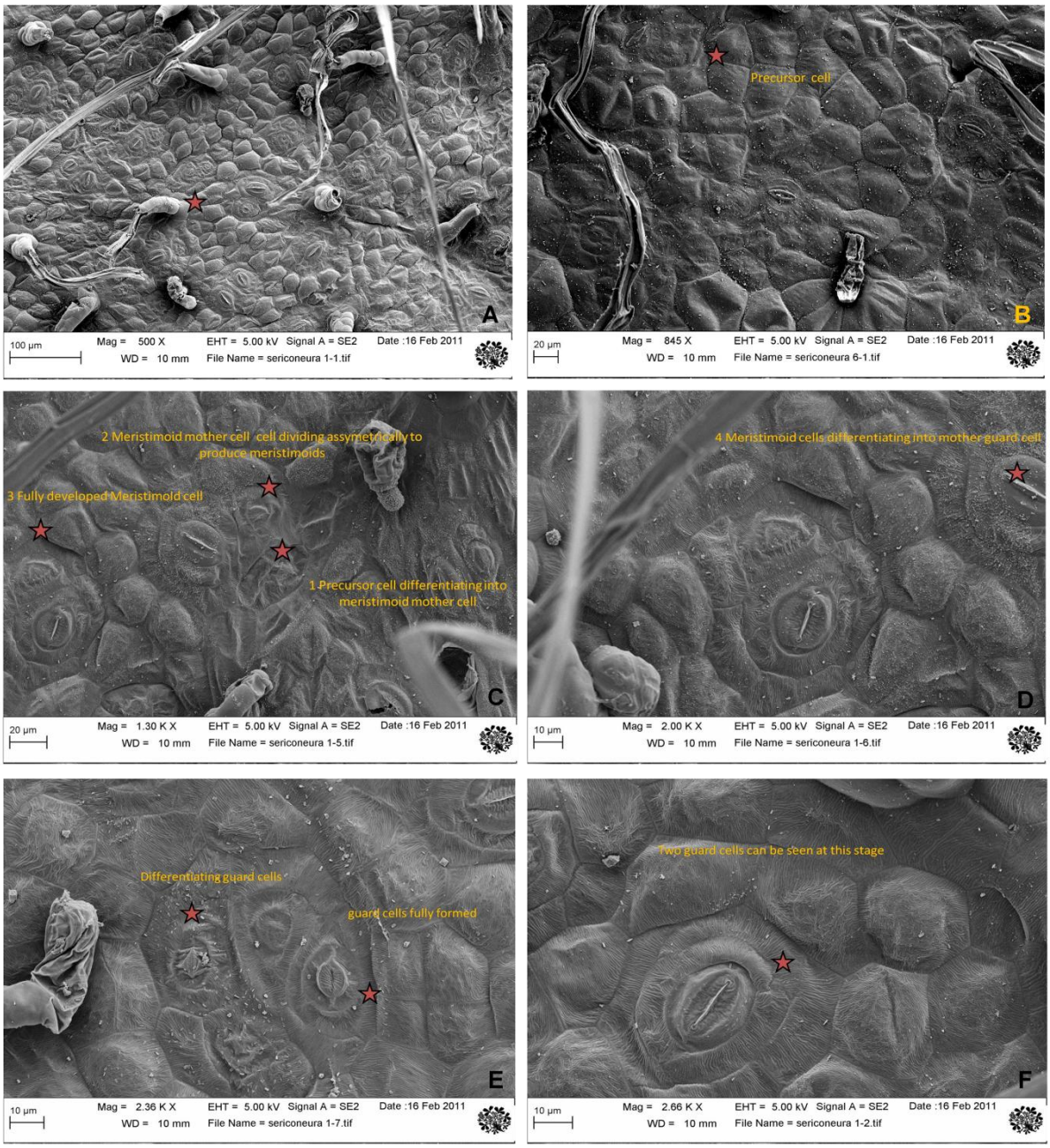

Figure 3.5: Development of stomatal clusters in *B. sericoneura*. (A) Stomatal development starts after trichomes have reached maturity (B) Living abaxial epidermis from developing leaf showing precursor cells that would differentiate into meristimoid mother cells (C) Meristimoid mother cell differentiating into meristimoids (D) Meristimoid cell differentiating into guard mother cells (E) Differentiating guard cells are fully formed guard cells (F) Fully developed stomata.

3.3.3.3 Traits related to trichome patterning

Begonia section Gireoudia varies for trichome form and density. Two kinds of trichomes are present on *Begonia* leaves - whiplash and glandular trichomes. Trichomes are present both on abaxial and adaxial sides of the leaf, leaf blades and also on leaf petioles in some of the species. A third kind of epidermal appendages known as scales are also present for some of the species. Figure 3.6, illustrates the three kinds of appendages found on the leaves of species in *Begonia* section Gireoudia and Table 3.7 describes the variation in densities of trichomes. The presence of these three kinds of appendages on *Begonia* leaves has also been reported by Burt- Utley (1985).

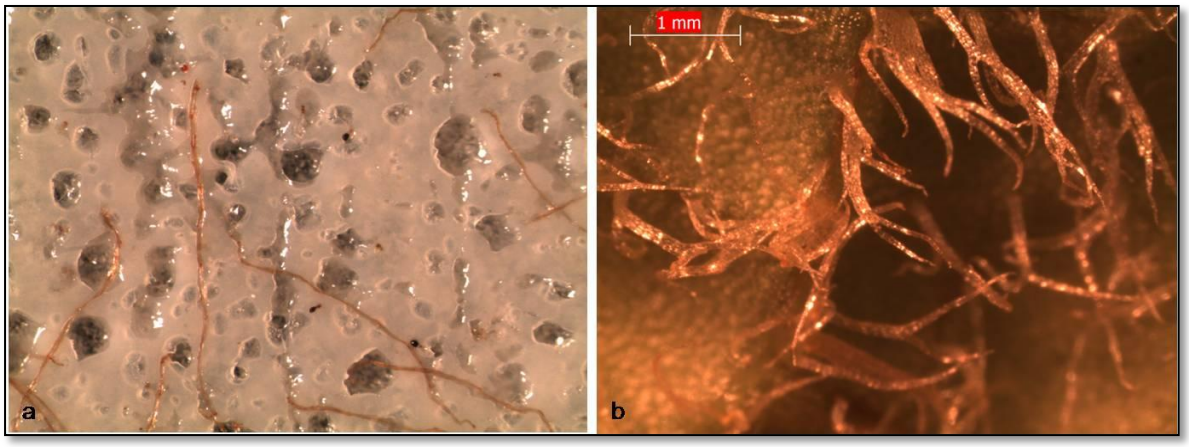

Figure 3.6: The three kinds of appendages present on the leaves in *Begonia* species in section Gireoudia. Figure 3.6 (a) illustrates the presence of whiplash and glandular trichomes on the leaves of *B. plebeja*. 3.6 (b) represents scales growing on the abaxial side of *B. imperialis*.

a. Abaxial side of the leaf

The abaxial sides of the leaves are generally denser with whiplash trichomes compared to the glandular trichomes. Density of whiplash trichomes on the abaxial side of the leaf varies from 0.6 (*B. stigmosa*) to 72.5 (B. *peltata*) in number, whereas glandular trichomes are not very frequent on the abaxial side of the leaf and are present only in few species such as *B. plebeja*

and *B. conchifolia*. They are missing for most species. *B. multistaminea* has the highest number of glandular trichomes on the abaxial side of the leaf. Length of whiplash and glandular trichomes also vary on the abaxial side of the leaf. *B. plebeja* produces the largest whiplash trichomes (0.16 mm). *B. multistaminae* produces the largest glandular trichomes (37.3 mm).

b. Adaxial side of leaf

The adaxial sides of the leaves are generally less dense for whiplash and glandular trichomes. The maximum numbers of whiplash trichomes on the adaxial side of the leaf was observed for *B. involucrata* (6.23). Glandular trichomes are not very frequent on the adaxial side of the leaf. They seem to be a distinguishing characteristic of *B. multistaminea* where they have a maximum density on both sides of the leaf. The length of whiplash and glandular trichomes also varies on the adaxial side of the leaf. *B. multistaminae* produces the largest whiplash trichomes (1.65 mm). *B. mazae* produces the largest glandular trichomes (0.18 mm).

Table 3.7: Illustrate a detailed summary of stomatal and trichome related traits in *Begonia* section Gireoudia. The number of replicates used is 2. The abbreviations for the traits measured are described as follows. Number of stomatal clusters per mm^2 (NscF), Average number of clusters (Av), substomatal cluster width & length (sscw & sscl), Number of stomata per cluster (Nsc), abaxial and adaxial type of trichomes (bTt and dTt), abaxial and adaxial length of whiplash trichomes (blt1 & dlt1), abaxial and adaxial number of whiplash trichome (bNt1 & dNt1), abaxial and adaxial length of glandular trichome (blt2 & dlt2), abaxial and adaxial number of glandular trichome (bNt2 & dNt2).

Specie	NscF	Av	sscw	sscl	Nsc	bTt	blt 1	bNt1	blt2	bNt2	dTt	dlt1	dNt1	dlt2	dNt2
B. mazae	139.10	0.18	0.11	0.12	3.03	1	0.70	2.50	0.02	0.17	1	0.65	4.83	0.18	0.83
B. lindleyana	18.80	0.17	0.07	0.12	1.00	1	0.32	15.0	Ab	0.00	1	0.68	9.33	Ab	0.00
B. purianata	41.10	0.13	0.07	0.07	2.20	0	1.38	23.33	0.01	0.33	0	0.52	8	0.01	0.16
B. peltata	205.0	0.14	0.08	0.07	2.40	1	0.23	72.50	Ab	0.00	1	0.25	29.1	Ab	0.00
B. carriae	225.8	0.13	0.06	0.06	1.63	1	1.60	31.75	Ab	0.00	1	0.30	38.0	Ab	0.00
B. conchifolia	128.3	0.19	0.08	0.07	2.00	0	0.59	25.67	0.04	1.00	0	0.58	3.06	Ab	0.00
B. plebeja	125.0	0.14	0.08	0.09	2.40	0	0.89	9.17	0.16	1.33	0	0.68	1.61	0.04	1.44
B.kellermani	218.3	0.11	0.07	0.08	2.60	1	0.31	45.33	Ab	0.00	1	0.11	2.67	Ab	0.00
B. sericoneura	204.1	0.12	0.07	0.07	2.00	1	0.94	49.33	0.04	10.0	3	1.02	15.3	0.05	3.67
B. corredorana	24.80	0.16	0.08	0.07	1.50	0	0.73	1.50	0.04	1.50	2	0.32	0.5	Ab	0.00
B. squarrosa	90.00	0.20	0.08	0.08	2.10	1	1.06	1.33	0.04	8.83	2	Ab	0	0.02	0.33
B. heracleifolia	197.5	0.46	0.28	0.23	3.20	1	0.57	4.67	0.04	1.50	1	0.61	5	0.04	1.33
B. multinervia	105.0	0.23	0.15	0.13	4.00	1	0.59	1.17	0.02	0.17	1	0.58	1.67	0.03	0.50
B.hydrocotylifolia	19.10	0.15	0.05	0.04	1.10	1	1.51	37.00	0.04	5.66	3	1.19	3.66	0.07	4.66
B. nelumbiifolia	164.1	0.13	0.07	0.07	1.00	1	1.05	4.50	Ab	0.00	1	0.93	2.17	Ab	0.00
B. carolineifolia	32.80	0.13	0.06	0.06	1.00	1	1.05	4.50	Ab	0.00	1	0.92	2.16	Ab	0.00
B. stigmosa	30.10	0.17	0.06	0.05	1.70	0	0.60	0.66	0.04	5.00	2	Ab	0	0.14	1.00
B. thiemie	375.80	0.10	0.10	0.05	1.00	1	0.22	1.67	0.01	0.33	1	0.27	5	0.12	0.17
B. multistaminae	200.0	0.14	0.09	0.08	2.80	0	0.46	1.00	0.08	37.3	0	1.65	2	0.06	8.33
B. involucrata	60.00	0.04	0.02	0.02	1.13	1	0.52	58.6	Ab	0.00	1	0.56	62.3	Ab	0.00
B. cardiocarpa	141.60	0.14	0.09	0.07	3.33	1	0.69	12.00	Ab	0.00	1	0.63	7.33	Ab	0.00

3.3.3.4 Traits related to vascularisation patterning

Begonias in section Gireoudia have a well developed vascular system. The anatomical features related to number and arrangements of vascular bundles were scored for comparative analysis. The species in *Begonia* section Gireoudia exhibited variation for vascularisation patterning traits of the petiole (Table 3.8). Figure 3.7, represents variation in the number and arrangement of vascular bundles for the 21 *Begonia* species.

The vascular bundles in *Begonia* section Gireoudia are arranged in two rings; an outer and inner ring. In some species such as *B. plebeja*, *B. carolinefolia* and *B. sericoneura* a single central bundle is also present.

The number of inner and outer vascular bundles varied among the species of the section Gireoudia. The average number of inner vascular bundle falls in the range of 5 to 11 while that of outer vascular bundles falls in the range of 8 to 36.

The inner and outer vascular bundles are more or less oval in cross section. The height of inner vascular bundles ranged from 0.1 mm (*B. plebeja* and *B. conchifolia*) to 0.42 mm (*B. nelumbifolia*). The inner vascular bundles also varied for width. The width ranges from 0.13 mm (*B. plebeja*) to 0.33 mm (*B. carolinefoli*a).

The height of outer vascular bundles ranged from 0.13 mm (*B. conchifolia*) to 0.39 mm (*B. nelumbifolia*). The width of outer vascular bundles also varies to an extent. The width of outer vascular bundles falls in the range of 0.11 to 0.26 mm. The largest value for the width of outer vascular bundles was for *B. plebeja* and the smallest value was for *B. carolinefolia*.

Table 3.8: Illustrate a detailed summary of traits related to vascularisation patterning in *Begonia* section Gireoudia. The number of replicates used is 2. Mean values are given for the traits including width of petiole (wp), Number of central vascular bundles (Ncvb), Number of inner vascular bundles (Nivb), Number of outer vascular bundles, (Novb), Length of inner vascular bundles (Livb), width of inner vascular bundles (wivb), Length of outer vascular bundles (Lovb), width of outer vascular bundles (wovb). The measurements are expressed in mm.

Specie	wp	Ncvb	Nivb	Novb	Livb	wivb	Lovb	wovb
B. mazae	2.92	0	5.50	17.50	0.32	0.18	0.22	0.14
B. lindleyana	2.98	1	5.50	26.50	0.25	0.16	0.20	0.12
B. purianata	4.29	1	5.00	26.50	0.32	0.18	0.25	0.16
B. peltata	4.62	0	8.00	35.50	0.39	0.26	0.31	0.20
B. carriae	2.87	1	5.50	9.00	0.38	0.20	0.27	0.11
B. conchifolia	2.08	0	5.00	9.33	0.19	0.10	0.13	0.06
B. plebeja	2.42	1	5.33	15.67	0.19	0.13	0.16	0.11
B.kellermani	2.51	0	6.50	9.50	0.28	0.16	0.21	0.13
B. sericoneura	4.82	1	7.50	29.50	0.34	0.19	0.30	0.15
B. corredorana	3.85	1	5.00	23.5	0.31	0.17	0.22	0.15
B. squarrosa	2.92	0	5.00	20.00	0.25	0.17	0.21	0.13
B. heracleifolia	5.82	1	6.50	20.00	0.31	0.31	0.31	0.19
B. multinervia	4.20	0	6.00	19.00	0.38	0.20	0.27	0.16
B. hydrocotylifolia	2.13	1	5.00	10.00	0.25	0.13	0.17	0.14
B. nelumbiifolia	5.87	0	10.50	33.50	0.42	0.24	0.39	0.19
B. carolineifolia	4.06	1	9.00	20.50	0.39	0.33	0.30	0.26
B. stigmosa	4.15	1	6.00	14.00	0.362	0.21	0.29	0.20
B. thiemie	4.28	0	6.50	27.50	0.42	0.27	0.34	0.21
B. multistaminae	4.28	1	6.00	25.00	0.30	0.26	0.21	0.13
B. involucrata	3.50	1	7.00	17.00	0.31	0.16	0.29	0.16
B. cardiocarpa	2.74	0	8.00	8.00	0.26	0.15	0.18	0.11

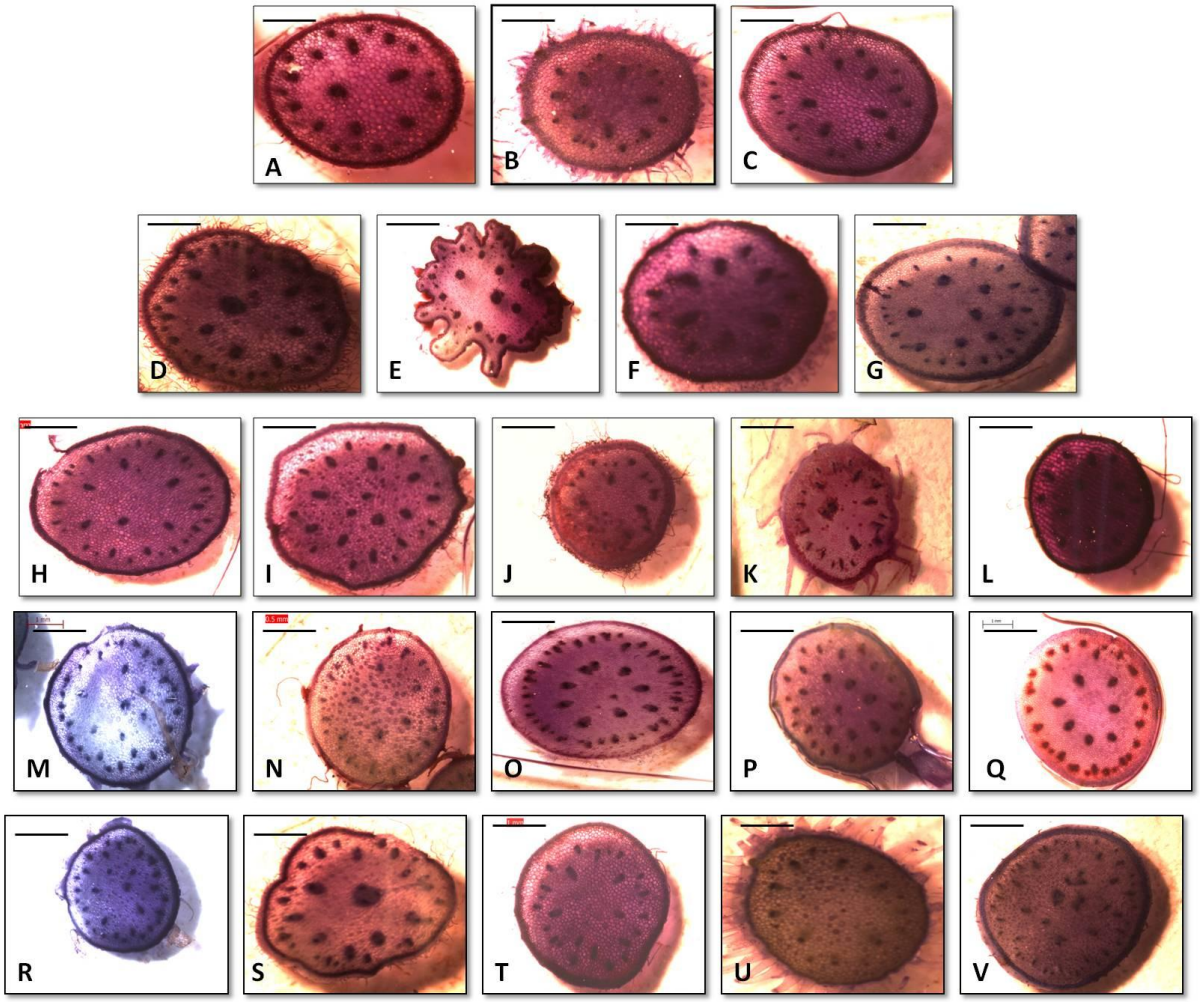

Figure 3.7: Vascularisation patterns for species in *Begonia* section Gireoudia including *B. Plebeja* (A), *B. imperialis* (B), *B. peltata* (C), *B. lindiliyana* (D) , *B. heracleifolia* (E), *B. Kellermani* (F), *B. multistaminae* (G), *B. puriniata* (H), *B. involucrata* (I) , *B. conchifolia* (J), *B. carrieae* (K) , *B. hydrocotilifolia* (L), *B. corredorana* (M), *B. squarrosa* (N) , *B. thiemie* (O), *B. mazae* (P), *B. carilinefolia* (Q), *B. stigmosa* (R), *B. Sericoneura* (S), *B. multinervia* (T), *B. cardiocarpa* (U), *B. nelumbifolia* (V). The images were generated by carrying out free hand petiole sections.

3.4 Leaf functional traits

Below are the results for the plant functional and resource use strategy traits. The traits scored in each category have been explained to a fuller detail with reference to the values globally found for other species.

a. Net Assimilation Rate

Net Assimilation rate (µmol m^{-2} s^{-1}) varies to a small extent among the species of *Begonia* section Gireoudia (Figure 3.8). On average species in section Gireoudia assimilate CO_2 at a rate of 2.75 µmol m^{-2} s^{-1} with a range of from 0.73 µmol m^{-2} s^{-1} (*B. lindileyana)* to 4.58 µmol m^{-2} s^{-1} (*B. thiemie)*. Table 3.9 presents a summary of mean net assimilation rate values and standard deviations for species in *Begonia* section Gireoudia. These values are well within the range of what has been observed in the global plant trait network (Glopnet) leaf data. Glopnet leaf data that presents a wide range of vegetation types varied for net assimilation rates and ranged between 1.14 umol m^2 s^{-1} and 42 umol m^2 s^{-1} (n = 812). The range of net assimilation rate values for the herbaceous plants (n = 155) observed in the global plant trait network falls in the range of 1 umolm^2s^{-1} to 42 umol m^2 s^{-1} (Wright et al., 2004).

Net assimilation rate observations for the species in *Begonia* section Gireoudia are at the lower range of values observed for the herbaceous plants in the global plant trait network data. These values are also at the lower range of values observed for tropical herbaceous plants. Herbaceous shade plants in tropical forests on average have net assimilation rates in the range of 5 umolm^2s^{-1} to 10 umolm^2s^{-1} whereas those in dry habitats are marked by net assimilation rate values between 15 umolm^2s^{-1} to 30 umolm^2s^{-1} (Larcher, 2003). For shade plants values as low as 2 umolm^2s^{-1} have also been observed. If we take into account the possibility that *Begonia* might be doing CAM then the values are well within the range of

CAM plants. In general CAM plants in the light have on average a net assimilation rate of 2 (5-12) umolm^2s^{-1} whereas in dark a CAM plant can carry out a net assimilation rate of (6-10) 20 umolm^2s^{-1} (Larcher, 2003).

b. Stomatal conductance

Stomatal conductance varies between 28.12 mmol m^{-2} s^{-1} (*B. nelumbifolia*) to 173.1 mmol m^{-2} s^{-1} (*B. carriea*e) (Figure 3.9). On average *Begonia* species in section Gireoudia have a stomatal conductance of 59.94 mmol m^{-2} s^{-1}. Table 3.8 presents a summary of mean stomatal conductance rates and standard errors for species in *Begonia* section Gireoudia. The stomatal conductances are much lower than what one would expected for herbaceous plants. Herbaceous shade plants in tropical forests on average have stomatal conductance values in the range of 6 to 200 mmol m^{-2} s^{-1} where as those in dry habitats are marked by 150 to 700 mmol m^{-2} s^{-1} (Larcher, 2003).

c. Total chlorophyll content

A survey was carried out to assess the amount of chlorophyll present in a range of *Begonia* (Figure 3.10). The chlorophyll content for South Asian *Begonias* ranged from 12.18 Spad units to 66.12 spad units (Table 3.11). Among the species in section Gireoudia the chlorophyll content varied from 21.39 spad unit for *B. carriae* to 50.67 spad units for *B. hydrocotilifolia* (Figure 3.10). On average *Begonia* species contained 33.4 spad unit of chlorophyll. The values exhibit sufficient overlap with terrestrial crop plant species e.g, rice (35.85), maize (57.45) and wheat (44.61) etc (Takebe and Yonayama, 1989; Wood et al., 1992, Follett et al., 1992).

d. Total Nitrogen content

Total nitrogen content varies among the species of *Begonia* section Gireoudia signifying differences in the amount of photosynthetic enzymes and nitrogen rich molecules (Figure 3.11). Among the section the total nitrogen content varies from 1.64% (*B. carrieae*) to 5.51% (*B. involucrata*). Table 3.9 presents a summary of total Nitrogen content and standard deviation for species in *Begonia* section Gireoudia. *Begonia* species in section Gireoudia has a mean nitrogen content of 3.03% in their leaves. The values exhibit sufficient overlap with herbs, grasses, and woody plants where the values for the total nitrogen content ranged from 0.2 to 6.4% (Wright et al, 2005). Total nitrogen content observations for the herbaceous plants (n = 371), in the global plant trait network data falls in the range of 0.78 % to 6.36 % (Larcher, 2003).

e. Total carbon content

Total carbon content also varies among the species of *Begonia* section Gireoudia signifying differences in the amount of investment a leaf has put in, in generating a new leaf (Figure 3.12). Among the section the total carbon content varies from 37.1% (*B. carrieae*) to 57% (*B. multinervia*). *Begonia* species in section Gireoudia has a mean carbon content of 50.62% in their leaves (Table 3.10).

f. Leaf mass Area

Leaf mass area varies among the species of *Begonia* section Gireoudia signifying differences in investment per unit photosynthetic leaf area (Figure 3.13). Among the section the leaf mass area varies from 4.9 g m^{-2} (*B. involucrata*) to 195 g m^{-2} (*B. hydrocotilifolia*). *Begonia* species in section Gireoudia has a mean LMA of 40.20 g m^{-2} (Table 3.10). Plant species growing in the field has also shown ample variation in LMA, with extreme values ranging from less than

3 g m^{-2} for freshwater *Myriophyllum* species (Gerber & Les, 1994) to > 2000 g m^{-2} for *Agave deserti* (Nobel, 1980). The LMA of herbaceous plants is comparatively low compared to trees and succulents and increases in the order of aquatic plants < ferns to < herbs/grasses < deciduous shrubs and trees < evergreen shrubs and trees and succulents (Poorter et al., 2009). The data in the literature shows that LMA of most herbaceous plants (n = 499) have values in the range of 14.4 g m^{-2} to 379.2 g m^{-2}. Most of the terrestrial species in the field lie between 30 g m^{-2} and 330 g m^{-2}. *Begonia* species are well within this range. However the values are skewed towards the lower end of the globally observed values.

g. Leaf dry matter content

Total leaf dry matter content varies among the species of *Begonia* section Gireoudia signifying differences in investment (Figure 3.14). *Begonia* species in section Gireoudia has a mean leaf dry matter content of 72.24 mg g^{-1} in their leaves. Among the section the total dry matter content varies from 21 mg g^{-1} *B. carrieae* to 224.9 mg g^{-1} (*B. hydrocotilifolia*). The leaf dry matter content values for the species in *Begonia* section Gireoudia are very low. At the global level, different functional groups in the field showed a wide variation in leaf dry matter content, with values ranging from164 mg g^{-1} to 414.7 mg g^{-1}. In general the lowest values are found for short lived forbs and the highest for trees (Wright et al., 2004).

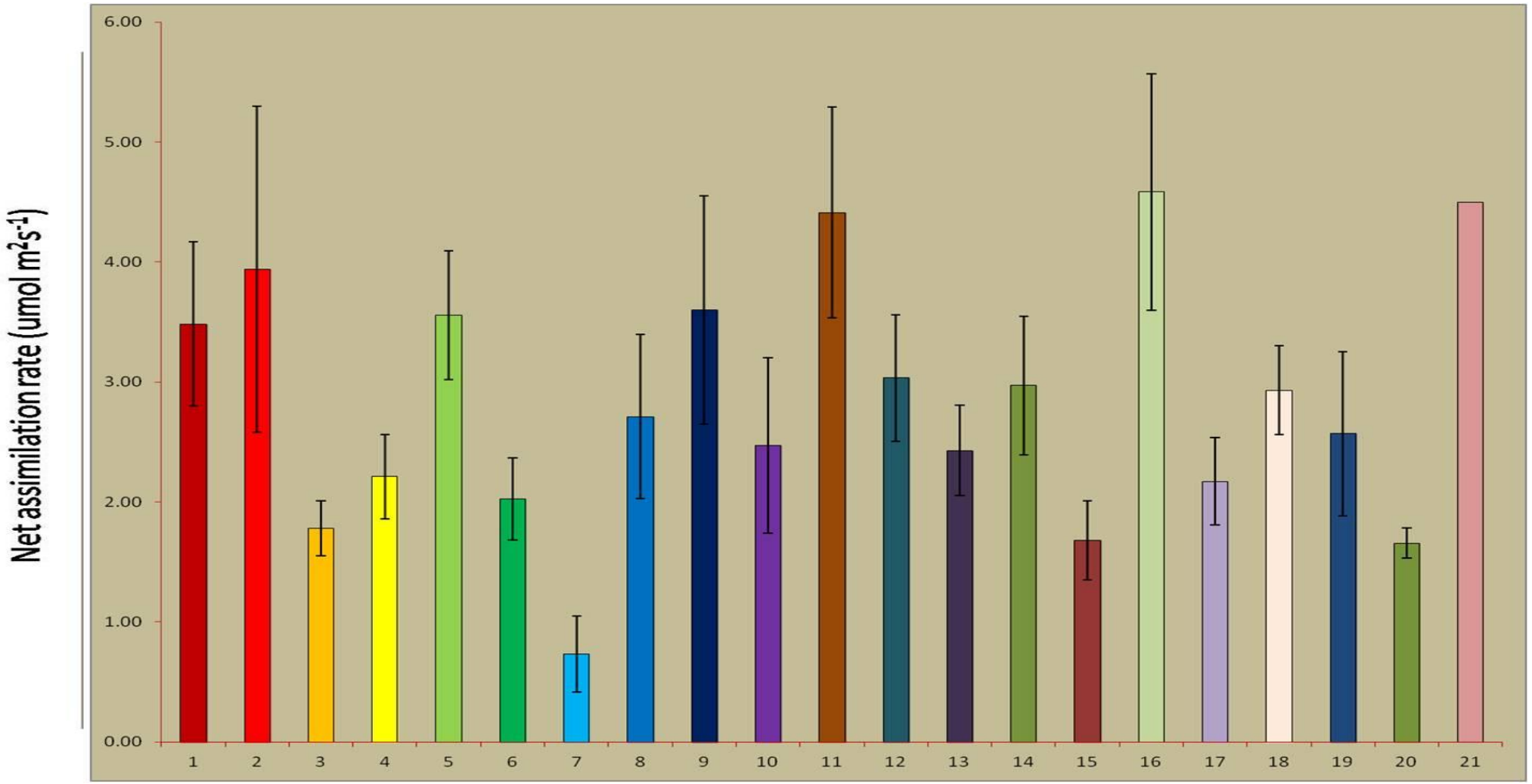

Figure 3.8. Mean net assimilation rates of 21 species of *Begonia* section Gireoudia. On the x axis 21 species of *Begonia* section Gireoudia are listed. 1=*B. carolinefolia*, 2=*B. carriae* , 3=*B.conchifolia*, 4=*B. corredorana*, 5= *B. heracleifolia*, 6= *B. hydrocotilifolia*, 7= *B. lindiliyana*, 8 = *B. mazae*, 9= *B. multistaminae*, 10= *B. nelumbifolia*, 11=*B. peltata*, 12= *B. plebeja*, 13= *B. sericoneura*, 14= *B. squarrosa*, 15=*B. stigmosa*, 16= *B. thiemie*, 17= *B. kellermani*, 18= *B. puriniata*, 19= *B. multinervia*, 20= *B. involucrata*, 21= *B. cardiocarpa*,. The error bars represents standard deviation.

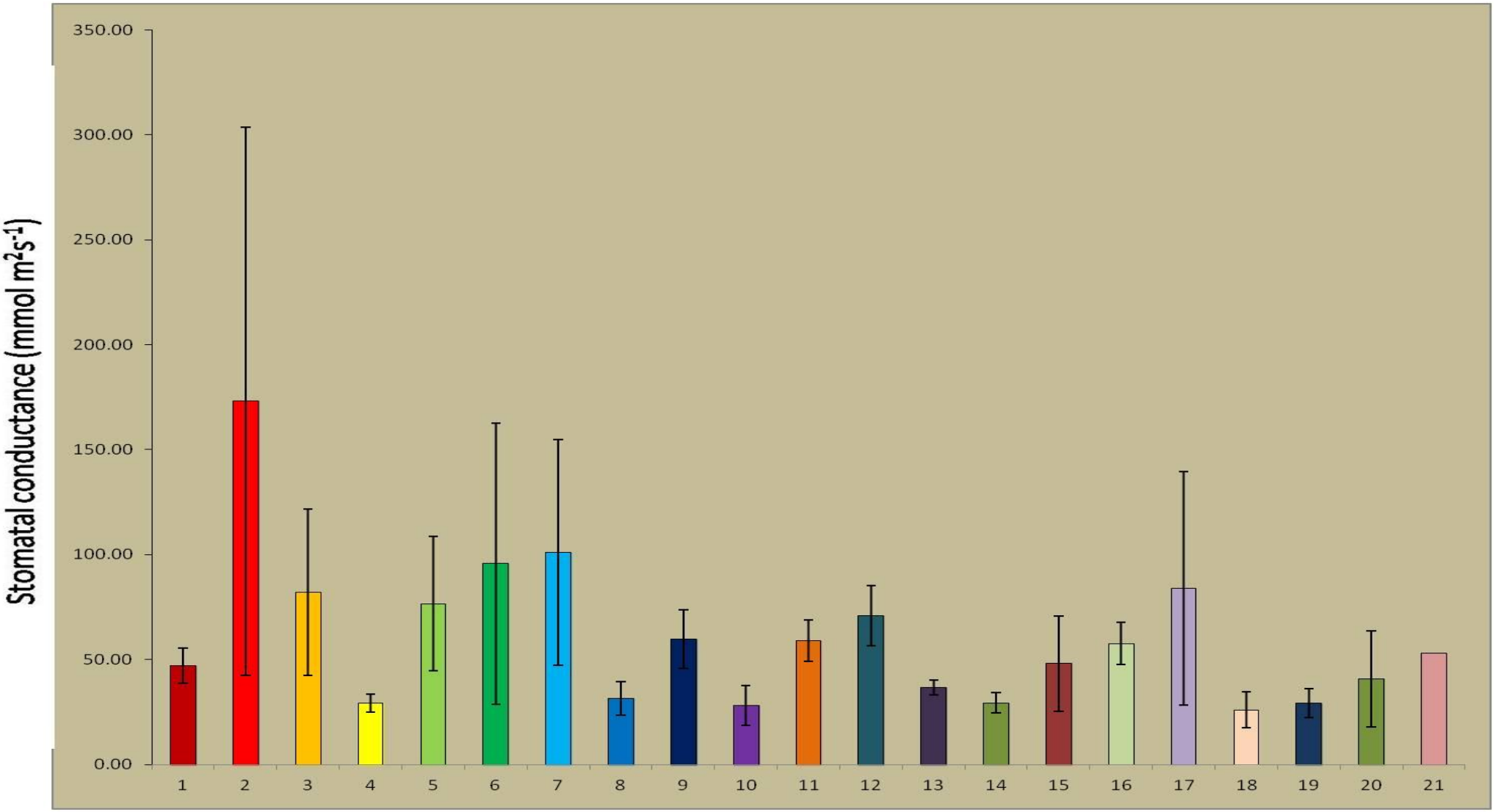

Figure 3.9: Mean stomatal conductance rates of 21 species of *Begonia* section Gireoudia. On the x axis 21 species of *Begonia* section Gireoudia are listed. 1=*B. carolinefolia*, 2=*B. carriae* , 3=*B.conchifolia*, 4=*B. corredorana*, 5= *B. heracleifolia*, 6= *B. hydrocotilifolia*, , 8 = *B. mazae*, 9= *B. multistaminae*, 10= *B. nelumbifolia*, 11=*B. peltata*, 12= *B. plebeja*, 13= *B. sericoneura*, 14= *B. squarrosa*, 15=*B. stigmosa*, 16= *B. thiemie*, 17= *B. kellermani*, 18= *B. puriniata*, 19= *B. multinervia*, 20= *B. involucrata*, 21= *B. cardiocarpa*. The error bars represents standard deviation.

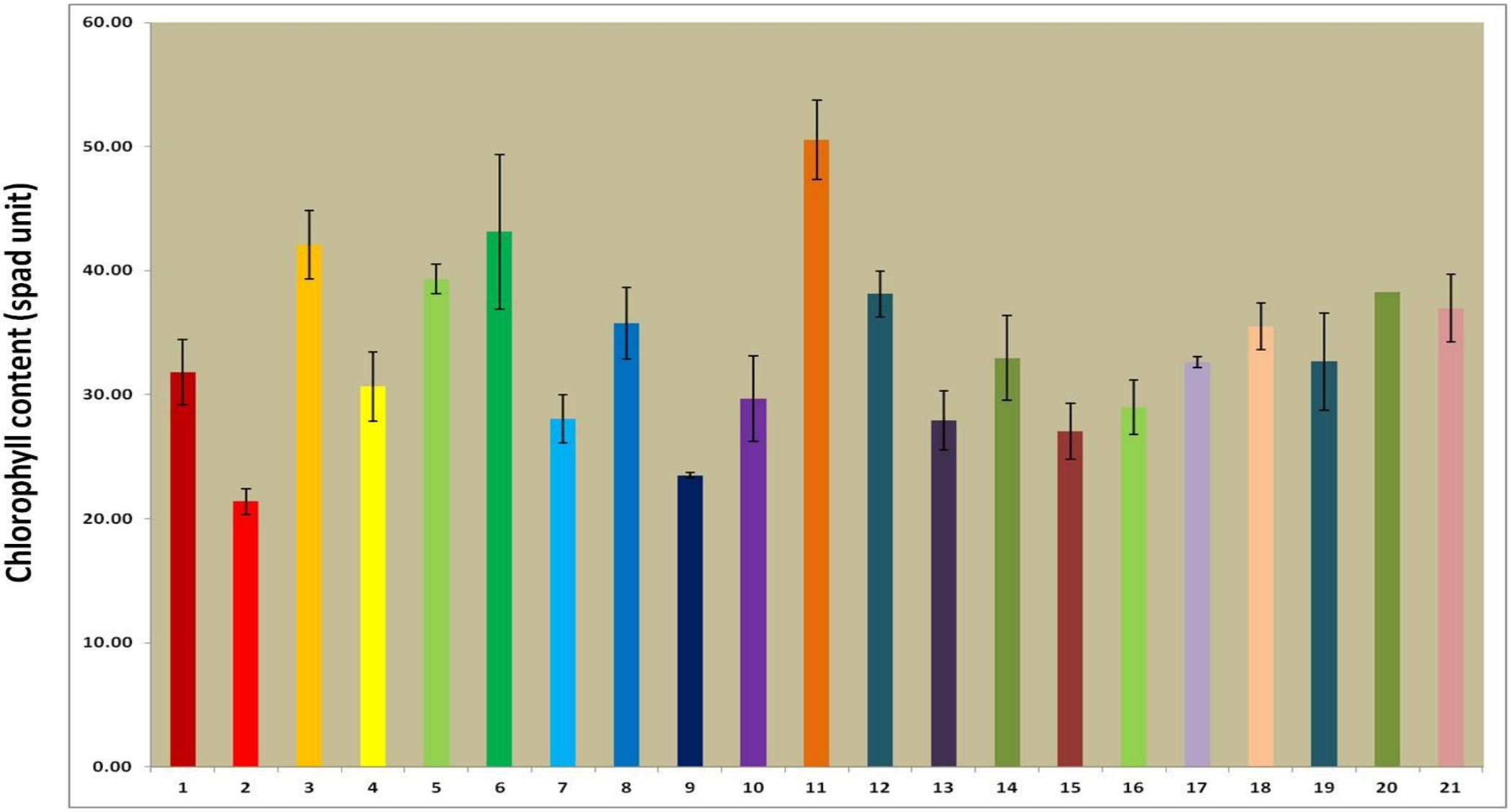

Figure 3.10: Mean chlorophyll content of 21 species of *Begonia* section Gireoudia. On the x axis 21 species of *Begonia* section Gireoudia are listed. 1= *B.carilinefolia*, 2= *B. carriae*, 3= *B.conchifolia*, 4= *B. corredorana*, 5= *B. heracleifolia*, 6= *B. hydrocotilifolia*, 7= *B. lindiliyana*, 8= *B. mazae*, 9= *B. multistaminae*, 10= *B. nelumbifolia*, 11= *B. peltata*, 12= *B. plebeja*, 13= *B. sericoneura*, 14= *B. squarrosa*, 15= *B. stigmosa*, 16= *B. thiemie*, 17= *B. kellermani*, 18= *B. puriniata*, 19= *B. multinervia*, 20= *B. involucrata*, 21= *B. cardiocarpa*. The error bars represent standard deviation.

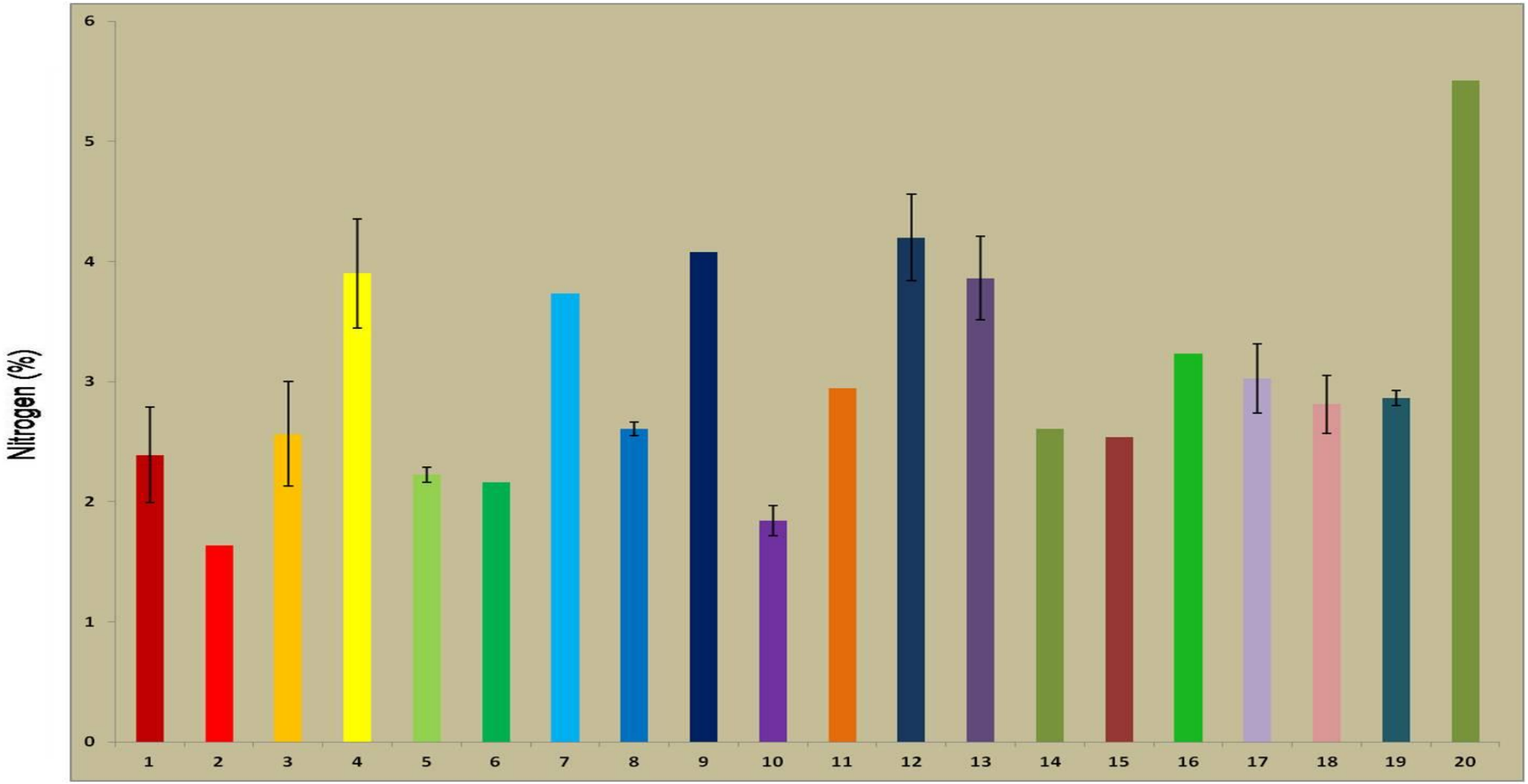

Figure 3.11: Mean nitrogen content of 20 species of *Begonia* section Gireoudia. On the x axis 20 species of *Begonia* section Gireoudia are listed. 1= *B.carilinefolia*, 2= *B. carrieae*, 3= *B. conchifolia*, 4= *B. corredorana*, 5= *B. heracleifolia*, 6= *B. hydrocotilifolia*, 7= *B. lindiliyana*, 8= *B. mazae*, 9= *B. multistaminae*, 10= *B. nelumbifolia*, 11 = *B. peltata*, 12 = *B. plebeja*, 13 = *B. sericoneura*, 14= *B. squarrosa*, 15= *B. stigmosa*, 16= *B. thiemie*, 17= *B. kellermani*, 18= *B. puriniata*, 19= *B. multinervia*, 20= *B. involucrata*. The error bars represent standard deviation.

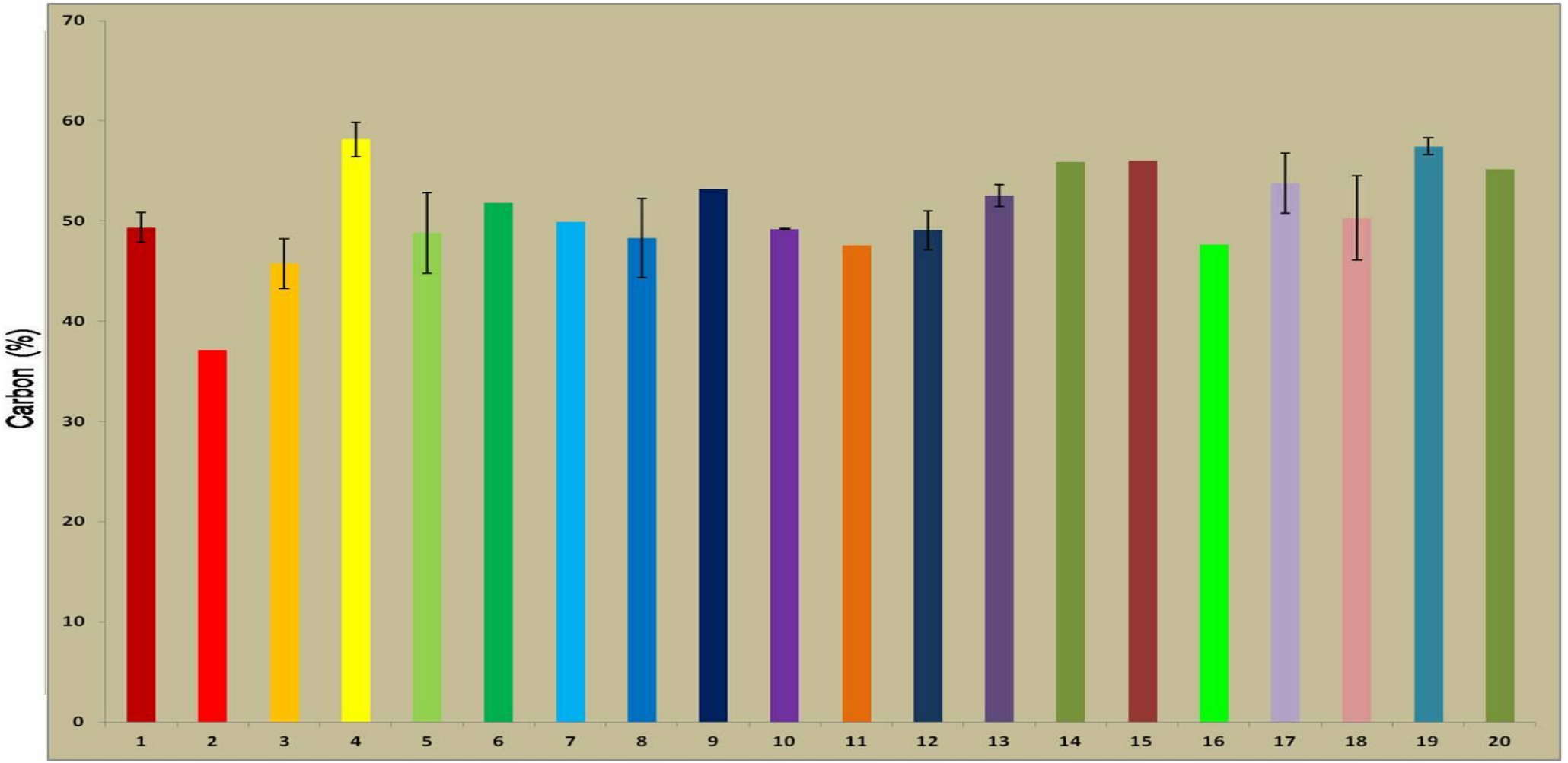

Figure 3.12: Mean carbon content of 20 species of *Begonia* section Gireoudia. On the x axis 20 species of *Begonia* section Gireoudia are listed. 1= *B.carilinefolia*, 2= *B. carrieae*, 3= *B. conchifolia*, 4= *B. corredorana*, 5= *B. heracleifolia*, 6= *B. hydrocotilifolia*, 7= *B. lindiliyana*, 8= *B. mazae*, 9= *B. multistaminae*, 10= *B. nelumbifolia*, 11 = *B. peltata*, 12 = *B. plebeja*, 13 = *B. sericoneura*, 14= *B. squarrosa*, 15= *B. stigmosa*, 16= *B. thiemie*, 17= *B. kellermani*, 18= *B. puriniata*, 19= *B. multinervia*, 20= *B. involucrata*. The error bars represent standard deviation.

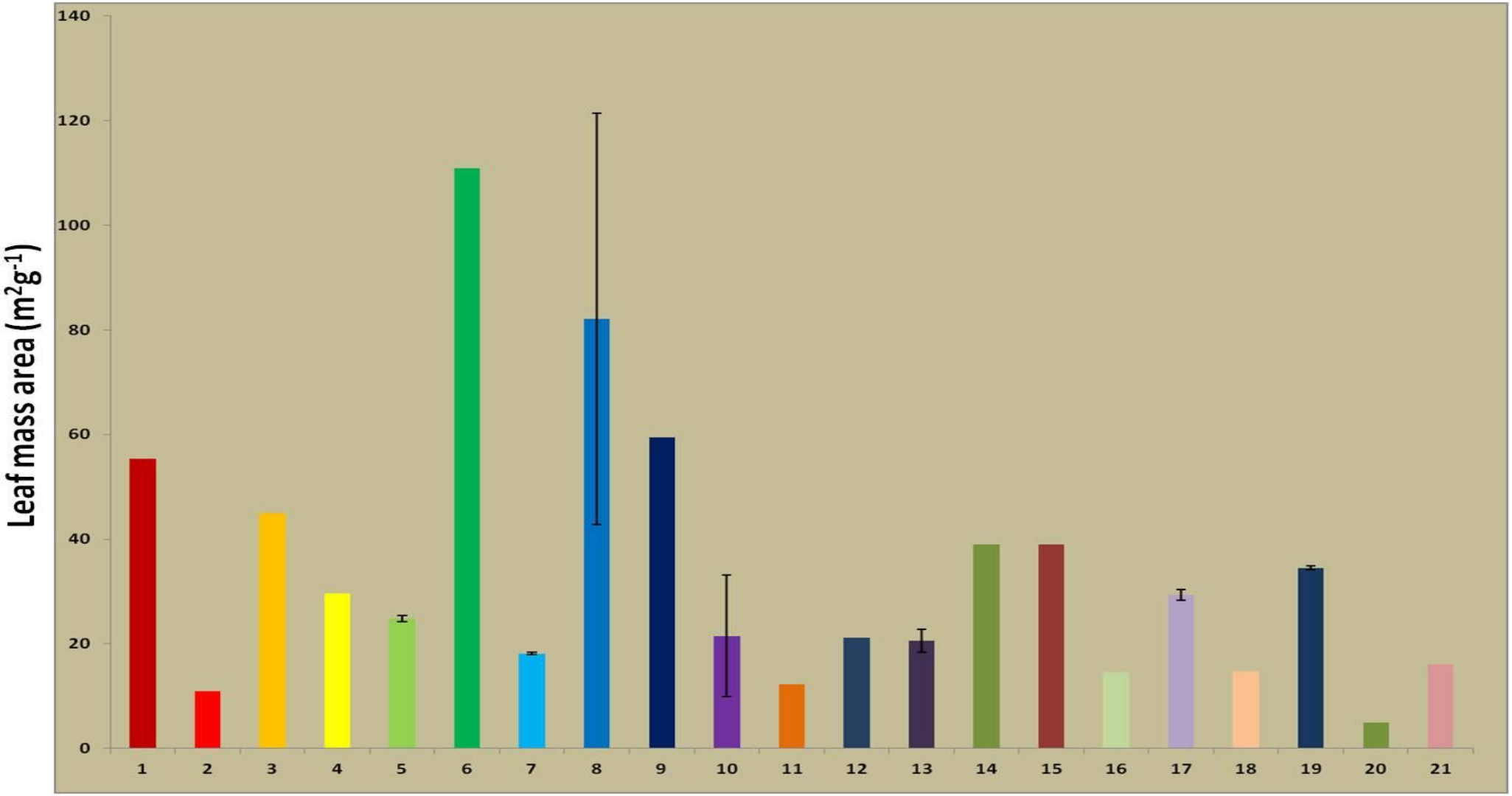

Figure 3.13: Mean leaf mass area of 21 species of *Begonia* section Gireoudia. On the x axis 21 species of *Begonia* section Gireoudia are listed. 1= *B. carolinefolia*, 2= *B. carriae*, 3= *B.conchifolia*, 4= *B.corredorana*, 5= *B. heracleifolia*, 6= *B. hydrocotilifolia*, 7 = *B. lindiliyana*, 8= *B.mazae*, 9= *B. multistaminae*, 10= *B. nelumbifolia*, 11= *B. peltata*, 12= *B.plebeja*, 13 = *B. sericoneura*, 14= *B. squarrosa*, 15= *B. stigmosa*, 16= *B. thiemie*, 17= *B.kellermani*, 18= *B. pruniata*, 19= *B. multinervia*, 20= *B. involucrata*, 21= *B.cardiocarpa*. The error bars represent standard deviation.

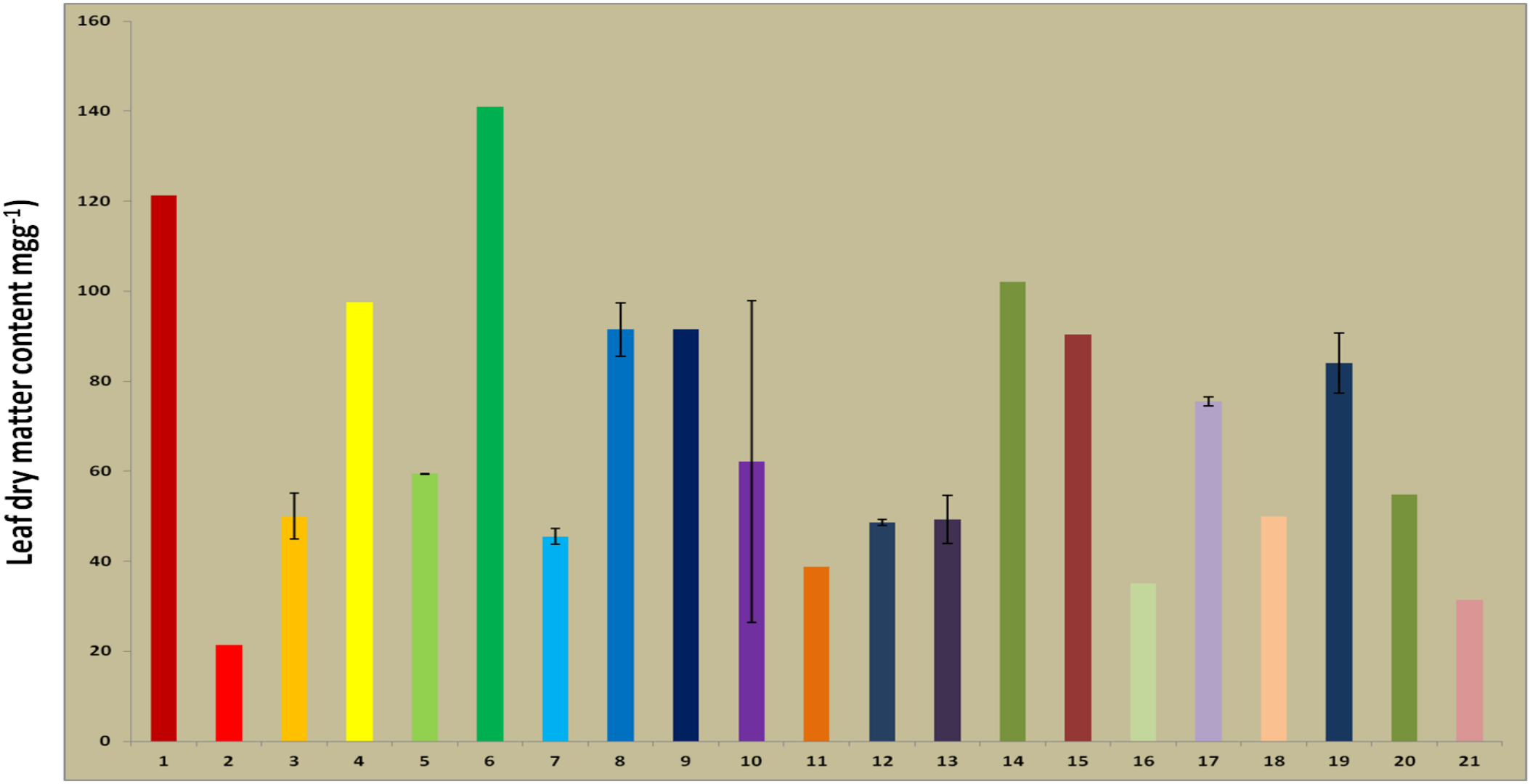

Figure 3.14: Mean leaf dry matter content of 25 species of *Begonia* section Gireoudia. On the x axis 23 species of *Begonia* section Gireoudia are listed. 1= *B. carolinefolia*, 2= *B. carriae*, 3= *B.conchifolia*, 4= *B.corredorana*, 5= *B. heracleifolia*, 6= *B. hydrocotilifolia*, 7 = *B. lindiliyana*, 8= *B.mazae*, 9= *B. multistaminae*, 10= *B. nelumbifolia*, 11= *B. peltata*, 12= *B.plebeja*, 13 = *B. sericoneura*, 14= *B. squarrosa*, 15= *B. stigmosa*, 16= *B. thiemie*, 17= *B.kellermani*, 18= *B. pruniata*, 19= *B. multinervia*, 20= *B. involucrata*, 21= *B.cardiocarpa*. The error bars represent standard deviation.

Table 3.9: Illustrate summary of functional traits in *Begonia* section Gireoudia. The traits measured included net assimilation rate (umolm^{2}s^{-1}), stomatal conductance (mmolm^{2}s^{-1}), chlorophyll (spad), and nitrogen content (%).

	Net assimilation rate			Stomatal conductance			Chlorophyll content			Nitrogen content			carbon		
List of species	**Mean**	**N**	**SD**	**Mean**	**N**	**SD**	**Mean**	**N**	**SD**	**Mean**	**N**	**SD**	**Mean**	**N**	**SD**
B. mazae	2.71	8	1.9	31.4	8	22.5	41.4	5	0.5	2.61	5	0.12	48.3	5	8.8
B. lindleyana	0.73	5	0.7	101.0	5	119.6	28.0	5	4.3	3.73	1	.	49.9	1	.
B. purianata	2.93	3	0.6	26.0	3	14.5	35.5	6	4.5	2.81	2	0.34	50.3	2	5.9
B. peltata	4.41	4	1.7	59.0	4	19.8	50.5	1	.	2.94	1	.	47.5	1	.
B. carriae	3.94	3	2.3	173.1	3	225.9	21.3	4	2.0	1.64	1	.	37.1	1	.
B. conchifolia	1.78	7	0.6	82.1	7	104.6	42.0	14	10.4	2.56	6	1.06	45.7	6	6.0
B. plebeja	3.03	19	2.3	70.9	19	62.0	38.1	21	8.4	4.2	11	1.19	49.0	11	6.3
B. kellermani	2.17	4	0.7	84.0	4	111.3	32.6	5	0.9	3.02	2	0.41	53.7	2	4.2
B. sericoneura	2.43	9	1.1	36.6	9	10.5	27.9	4	4.7	3.86	4	0.7	52.5	4	2.1
B. corredorana	2.21	4	0.7	29.1	4	8.6	30.6	6	6.8	3.9	2	0.64	58.1	2	2.4
B. squarrosa	2.97	6	1.4	29.3	6	11.5	32.9	4	6.8	2.6	1	.	55.8	1	.
B. heracleifolia	3.56	8	1.5	76.5	8	90.2	39.3	6	2.8	2.22	2	0.09	48.8	2	5.6
B. multinervia	2.57	3	1.1	29.1	3	11.9	23.5	2	<0.001	2.86	2	0.09	57.4	2	1.2
B. hydrocotilifolia	2.02	4	0.8	95.6	4	133.9	50.6	2	8.1	2.16	1	.	51.8	1	.
B. nelumbiifolia	2.47	5	1.6	28.1	5	20.9	27.9	2	7.2	1.84	2	0.18	49.2	2	<0.001
B.carolineifolia	3.48	7	1.8	47.2	7	22.1	31.8	6	6.4	2.39	2	0.56	49.3	2	2.1
B. stigmosa	1.68	5	0.7	48.0	5	50.6	27.0	4	4.5	2.54	1	.	56.0	1	.
B. thiemie	4.58	6	2.4	57.6	6	24.7	29.0	5	4.8	3.23	1	.	47.6	1	.
B. multistaminae	3.6	3	1.6	59.7	3	23.9	23.5	2	0.3	4.08	1	.	53.1	1	.
B. involucrata	1.65	2	0.1	40.7	2	32.4	38.2	1	-	5.51	1	.	55.2	1	.
B. cardiocarpa	4.5	1	.	53.1	1	.	36.9	2	3.8	.	.	.	.	.	.

Table 3.10: Summary of resource use traits in *Begonia* section Gireoudia. The traits measured included dry matter content (mg g^{-1}) and leaf mass area (g cm^{-2}).

	Leaf dry matter			Leaf mass area		
	Mean	**N**	**SD**	**Mean**	**N**	**SD**
B. mazae	91.51	3	10.2	82.08	3	68.0
B. lindliyana	37.43	2	14.0	15.72	2	4.20
B. purianata	47.06	2	3.9	17.49	2	3.9
B. peltata	38.78	.	.	12.17	.	.
B. carriae	21.38	.	.	10.88	.	.
B. conchifolia	50.00	6	12.3	44.91	6	14.1
B. plebeja	48.68	12	2.3	21.23	12	4.0
B. kellermani	75.52	2	1.4	29.29	2	1.4
B. sericoneura	49.32	4	10.7	20.57	4	4.4
B. corredorana	97.55	.	.	29.68	.	.
B. squarrosa	102.07	.	.	38.92	.	.
B. heracleifolia	59.39	2	0.08	24.77	2	0.7
B. multinervia	84.03	2	9.4	34.50	2	0.4
B. hydrocotylifolia	224.95	2	62.2	195.75	2	33.0
B. nelumbiifolia	62.14	2	50.4	21.50	2	16.3
B. carolineifolia	121.25	.	.	55.42	.	.
B. stigmosa	90.31	.	.	39.00	.	.
B. thiemie	35.02	.	.	14.57	.	.
B. multistaminae	91.48	.	.	59.498	.	.
B. involucrata	54.79	.	.	4.997	.	.
B. cardiocarpa	31.35	.	.	15.987	.	.

Table 3.11: Chlorophyll content values in single accessions of Asian, African and American *Begonias*. Chlorophyll content was measured by spad meter and the unit of measurement is spad.

Species	Mean	Origin	Species	Mean	Origin
B. tayabensis	35.9	Asia	***B. timorensis***	40.1	Asia
B. sutherlandii	16.65	Africa	***B. petasitifolia***	39.48	America
B. dregei	15.04	Africa	***B. humilis***	45.78	America
B. oxyloba	27.28	Africa	***B. wrightiana***	38.88	America
B. poculifera	57.42	Africa	***B. bissei***	41.86	America
B. ampla	34.64	Africa	***B. cucullata***	24.72	America
B. polygonoides	27.82	Africa	***B. glandulifera***	66.12	America
B. tricornis	24.96	Asia	***B. minor***	19.16	America
B. venusta	31.06	Asia	***B. grandis***	27.4	Asia
B. convolvulacea	20.08	America	***B. dipetala***	34.62	Asia
B. decora	25.28	Asia	***B. acutifolia***	41.94	Asia
B. chloroneura	25.64	Asia	***B. formosana***	12.18	Asia
B. pavonia	16.84	Asia	***B. palmata***	30.62	Asia
B. nigritarum	23.1	Asia	***B. tomentosa***	26.88	America
B. odorta	29.7	America	***B. cristata***	42.88	Asia/Americ
B. vicina	25.34	America	***B. brevirimosa***	32.8	Asia
B. rex	32.1	Asia	***B. masoniana***	19.42	Asia
B. reniformis	29.06	America	***B. obscura***	26.34	America
B. hispeda	20.34	America			
B. purpurea	28.14	America			
B. herbacea	57.26	America			
B. acetosa	37	America			
B. reniformis	35.44	America			
B. listada	37.04	America			

3.5 Conclusions

The results revealed that leaves in *Begonia* section Gireoudia displayed wide ranges in structural features. Variations are prominent in, for example, length and width of rhizomes as well as linear dimensions of leaf size such as leaf length and width as well as dissections of the leaf margins. The range of interspecific morphological variation for rhizome length and leaf size attributes is in agreement with the field observations of Burt-Utley (1985).

Species in *Begonia* section Gireoudia appear to have adaptations to absorb light in the understory shade of tropical forests. A red coloured abaxial leaf surface is observed in *B. multinervia,* while a shinning upper leaf surface is often seen in *B. conchifolia and B. hydrocotilifolia.* The abaxial red surface of the leaf has a greater potential of absorbing photosynthetic photon flux density (90% PPFD) compared to the green abaxial surfaces (82% PPFD) (Lee & Graham, 1986). Anthocyanin pigmentation has also been observed in different layers of leaf in *Begonia* species such as incase of *B. mazae* (Figure 3.4). The presence of anthocyanin layers inside the leaf has also been considered to be advantageous for reflecting photosynthetically useful light. The reflected light passes the chloroplast layer twice which increases the chance of each ray hitting a chloroplast (Lee and Stone, 1979).

Species in the section have also been observed to have lens-shaped epidermal cells that have long been thought to have a role in the increased absorption of diffuse light. In this survey, lens cells are more often observed in species that are exposed to direct sunlight in the tropical forests. Based on this we can conclude that the lens shaped epidermal cells in section Gireoudia might have a role in focusing of direct light. The lens shaped epidermal cells could also be beneficial in terms of storing water in the drier habitats to avoid the risk of collapse in less humid periods. These cells have also been shown to increase water repellency and thus improving the hydrophobic nature of the leaf surface (Vogelmann et al., 1996).

Although the leaves of species in *Begonia* section Gireoudia vary widely in their size, shape, succulence and thickness, their basic leaf anatomy is the same and includes an epidermis, multiple layered hypodermis and a mesophyll. The multiple layered hypodermis is basically a water-containing tissue immediately above and below the photosynthetic mesophyll and is a characteristic feature of *Begonia* species. It has also been reported in other plants such as *Peperomia* (Horner, 2012). Since species in *Begonia* section Gireoudia grow mainly epiphytically and sometimes terrestrially under low light in moist as well as sometimes in dry conditions, water shortage could be a serious issue. The presence of a multilayered hypodermis among the species in *Begonia* section Gireoudia could be related to water-storage and might be an adaptation to survive the few short months when rainfall becomes very low (Solereder and Meyer 1929).

The stomata in *Begonia* section Gireoudia appear to be more or less regularly distributed on the lower surface and also occur in groups as clusters. The stomata are always of the anisocytic type. *Begonia* stomatal development also shares many similarities with developmental processes in other plants such as *Arabidopsis*. The SEM scan of a juvenile leaf of *B. sericoneura* suggested that the amplifying asymmetric divisions of meristemoids occur normally in *Begonia,* so that a mature stoma is surrounded by small non-stomatal cells, just like in *Arabidopsis*. On the other hand, cells differentiating as stomata are clustered like patches of islands. Based on these phenotypes, we can conclude that the MMC (meristemoid mother cell) state is extended in *Begonia.*

The current study also revealed that *Begonias* exhibit highly interesting leaf economics. Net assimilation rate and stomatal conductance values varied to a small extent among the species of *Begonia* section Gireoudia. However, the values are very low. This suggests that there could also be a possibility that *Begonias* might be doing CAM. The values observed for

species in *Begonia* section Gireoudia are well within the range of CAM plants. However in a recent CAM study conducted on seven *Begonia* species by Johnston (pers. comm), no significant increase in total acid or malic acid content was observed between dawn and dusk foliage samples from the sampled species. This should be further investigated since malate fluctuations could have been masked by the particularly high acidity of the *Begonia* leaves. Importantly, all seven sampled species exhibited nocturnal stomatal opening, highlighting potential for assimilating carbon dioxide at night. Another important finding in that study was the high carbon isotope ratio of *B. ampla*, suggestive of the fact that this species could use CAM to an extent.

Other features such as investment of carbon and nitrogen substrates also displayed a wide range of variation. For example, differences were observed in the amount of invested energy in the leaf expressed as dry weight per unit area of leaf surface or leaf mass area. It appears that the studied species in *Begonia* section Gireoudia generally have much thinner leaves and invest less energy in photosynthetic tissue. The LMA values ranged from 4.9 gm^{-2} to 195 gm^{-2}. The values for LMA are well within the range of what is observed globally for herbaceous plants (14.4 gm^{-2} to 379.2 gm^{-2}). However the values were skewed towards the lower range of globally observed values. One possible explanation of such low LMA species in section Gireoudia could be their exposure to lower daily photon irradiance (DPI) in the glass house conditions as well as in the field conditions i.e in understory shade of tropical forests (Garnier and Freijsen, 1994). The low light intensities would restrict the plant to invest a larger amount of energy in the leaf. The leaf dry matter content values are also very low which could be explained by the presence of the multiple water filled hypodermis. Only a small proportion of the leaf consists of the mesophyll.

In this study of species level variation in section Gireoudia, species differed in ecophysiology and in morphological traits providing evidence that section Gireoudia speciated through habitat adaptation to specific environments.

CHAPTER 4. Adaptation of *Begonia* species to sun and shade habitats

4.1 Introduction

Based on their tolerance to sun and shade, plants can be grouped into sun and shade species. Each group has a particular suite of traits which increases photosynthetic efficiency in that habitat. The features that distinguish sun and shade species include variation in plant form, cellular anatomy, physiology and resource allocation and growth patterns.

Generally speaking, the leaves of plant species that are adapted for full sun (a) have significantly smaller surface area than leaves from typical shade plants (Larcher 2003). The smaller leaf proves to be advantageous in dry habitats because it offers a smaller boundary layer and better convective heat exchange with the environment. Dissected or compound leaves are more common in dry habitats. Generally speaking a highly dissected leaf has a thinner boundary layer that results in better convective heat exchange (Nobel, 1983; Schuepp, 1993).

Sun leaves are usually thicker than shade leaves with generally larger spongy and palisade mesophyll layers as well as thicker non-cellular waxy coatings. Such leaves are usually characterised by a lower proportion of chlorophyll *b* relative to that of chlorophyll *a*, a higher content of total chlorophyll proportional to fresh weight, and higher Ribulose bisphosphate carboxylase activity (Boardman, 1977). Another feature that characterises leaves of sun plants is that they usually have more stomata per unit surface area (Larcher, 2003).

4.2 Aims of the project

The aim of this study was to examine the structural features and physiological differences between *B. plebeja* and *B. conchifolia* plants in the context of the different light environments they are found in. The species are phylogenetically close and can be crossed to produce fertile and vigorous hybrids. Both the species were scored for morphological, leaf shape and area attributes, as well as functional and anatomical traits. The species were then compared for traits such as habit type, chlorophyll content, dry weight per unit surface area, and leaf thickness. Analysis of this data will help us determine whether or not they exhibit the structural features that typify sun or shade plants. It will also help us understand the kind of tradeoffs that have influenced the evolution of the traits involved in adaptation and have resulted in distinct distributions of the two species.

4.3 Results

4.3.1 Morphological differences between *B. plebeja* and *B. conchifolia*

Both the species were scored for qualitative and quantitative morphological variation. Given below is a description of the qualitative and quantitative differences for morphological traits between both the species (Table 4.1).

a. Anthocyanin pigmentation

A striking difference between both the species is in the production of anthocyanins. *B. plebeja* leaves have anthocyanin expression at the petiole attatchment point (‘red eye’), at their margins and in ‘blotches’ on the lamina between the major veins. *B. plebeja* also accumulates anthocyanins in the petiole. Conversely, anthocyanins are absent in *B. plebeja* stipules but present in *B. conchifolia* stipules.

b. Rhizome number and architecture

Both the species are rhizomatous. The rhizomes are repent and are sparingly branched. However differences in rhizome architecture arise from differences in size related rhizome traits. *B.plebeja* has periodic rhizome growth whereas growth in *B. conchifolia* is more or less continuous. *B. plebeja* is deciduous in some populations and in most habitats the plants are dormant for at least three months in the year (Burt-Utley, 1985). *B. plebeja* leaves are supported by large and thick rhizomes (125 mm x 16.9 mm) while *B. conchifolia* leaves are supported by rhizomes that are much smaller in size and are thin (46.22 mm x 7.4 mm).

c. Leaf production and size related traits

B. plebeja has much larger petioles than *B. conchifolia*. Leaf production and size related traits of leaves also varied between both the species. *B. plebeja* produces fewer leaves per cm length of its rhizome (1.42) compared to *B. conchifolia* (2.86) (calculating the number of scars as well as leaves present on the plant). Another striking difference between both the species is in their leaf morphology. The leaves in case of *B. plebeja* leaves are deciduous in most of the populations while those in *B. conchifolia* are persistent. *B. plebeja* leaves are thinner and bigger compared to *B. conchifolia* that produce smaller, thicker, more succulent leaves. Also *B. plebeja* produces non peltate leaves compared to *B. conchifolia* that produces peltate leaves.

Table 4.1: Qualitative and quantitative morphological differences between *B. plebeja* and *B. conchifolia*. The data was generated using measurements from two accessions of each species; hence the values present an average. The plants grew in the glass houses of Royal Botanic garden Edinburgh under same environmental conditions. The length and width of rhizomes are measured in mm.

Traits	***B. plebeja***	***B.conchifolia***
Colour of stipules	green or white	pink-red or red-pink
Colour of rhizome	no red pigment	presence of red pigment
Colour of petiole	faint pink	red / pink red
Colour red eye	red	white
Ab/pr blotch	yes	no
Margin	weak	absent
Habit	rhizomatous repent	rhizomatous repent
Simple vs compound leaf	simple	simple
Number of peltate	no	yes
Total number of rhizomes	10	9
Average length of rhizomes	125	46.22
Average width of rhizomes	16.9	7.4
Average number of scars	137	48
Total number of leaves	40	84
Total number of scars+leaves	177	132
Total number of scars+leaves/ length (cm) of rhizome 1	0.14	0.16

4.3.2 Leaf shape and area attributes differences

To quantitatively assess differences for leaf shape and area attributes between *B. plebeja* and *B. conchifolia*, leaves were collected and processed with lamina software (Bylesjo et al., 2008). A total of twelve quantitative traits were harvested using lamina software. A t test was then carried out to assess the significance of differences between the species. Results showed that significant differences are found for most of the traits captured in this category. Table 4.2 represents mean values, samples size (n), t and probability values (p) for *B. plebeja* and *B. conchifolia* leaf size and shape attributes.

a. Leaf Area

Significant variation for leaf area exists between both the species. *B. plebeja* has significantly bigger leaves with an average leaf area of 8848.6 mm^2 (n= 18, sdev = 5262.1) compared to *B. conchifolia* that produces small leaves with an average leaf area of 2081.6 mm^2 (n= 10, sdev = 900.1).

b. Perimeter to area ratio

The perimeter to area ratio describes variations in leaf shape. Among the species in section Gireoudia, the perimeter to area ratio is small for *B. conchifolia* (11.22 (n= 10, sdev = 0.44)), confirming a typically entire margin, whereas *B. plebeja* more serrated and occasionally small lobes, giving a higher perimeter to area ratio (16.17 (n= 18, sdev=1.78)).

b. Circularity index

The circularity index for *B. plebeja* leaf is 82.19 (n= 18, sdev = 1.78) while that for *B. conchifolia* leaf is 89.40 (n= 10, sdev = 6.38) illustrating that *B. conchifolia* leaf is much more circular than *B. plebeja.*

c. Length and width

The difference in the length of *B. plebeja* and *B. conchifolia* leaf is three fold with *B. plebeja* having an average leaf length of 123.7 mm (n= 18, sdev= 38.19) compared to *B. conchifolia* that has a length of 59.68 mm (n= 10, sdev = 13.32). Significant differences are also found for leaf width. The width of *B.conchifolia* leaf is almost half of that of *B. plebeja* with *B. plebeja* having a value of 100 mm (n= 18, sdev = 31.47) and *B. conchifolia* that of 47.12 mm (n= 10, sdev = 10.83).

e. Number of serrations

B. plebeja leaf has a higher number of indents then *B. conchifolia*. The difference is almost four fold with *B. plebeja* having mean indent number of 28.83 (n= 18, sdev = 9.37) and *B. conchifolia* having a mean indent number of 9.80 (n= 10, sdev = 3.99).

f. Indent depth mean:

Indent depth mean differs between the species. The indents in case of *B. plebeja* leaves are on average 11.18 mm deep (n= 18, sdev = 1.47) whereas the indents in *B. conchifolia* leaf are on average 9.32 mm deep (n= 10, sdev = 1.41).

g. Indent width mean:

Indent width mean differs between the species. The indents in case of *B. plebeja* leaves are an average of 1.76 mm wide (n= 18, sdev = 0.3) whereas the indents in *B. conchifolia* leaves are on average 0.68 mm wide (n= 10, sdev = 0.09).

g. Horizontal and vertical symmetry:

The species are identical in their horizontal symmetry. However significant differences are observed for vertical symmetry. *B. plebeja* leaves are vertically more symmetrical with a

value of 1.04 mm (n= 18, sdev = 0.09) compared to *B. conchifolia* with a value of 0. 97 mm (n= 10, sdev = 0.07).

Table 4.2: Significance of differences for leaf size and shape traits between *B. plebeja* and *B. conchifolia*. Mean, samples size (n), t and probability values (p) for the traits are also given. The leaf shape and area attributes were measured using lamina software. The traits were measured in mm.

Trait	*B. plebeja*					*B. conchifolia*						
	Mean	**N**	**SD**	**Min**	**Max**	**Mean**	**N**	**SD**	**Min**	**Max**	**t**	**P**
Area of Lamina	8848.6	18	5262.1	2140.3	1925.8	2081.6	10	900.1	1127.3	3498.4	4	0.0004
Perimeter	360.3	18	111.8	181.3	555.2	149.7	10	32.5	110.7	196.2	5.77	4.36E-06
Squared perimeter / Area	16.17	18	1.78	11.5	19.6	11.22	10	0.44	10.8	12.2	8.6	4.62E-09
Circularity	82.19	18	1.78	74.1	93.3	89.4	10	6.38	83.6	98.4	-3	0.006
Width of leaf	100	18	31.47	54.1	152.6	47.12	10	10.83	34.6	62.8	5.11	2.48E-05
Length of leaf	123.7	18	38.19	64.2	190	59.68	10	13.32	45.9	83.1	5.11	2.62E-05
Length : width	1.25	18	0.12	1	1.41	1.27	10	0.09	1.11	1.34	-0.5	0.61
Horizontal symmetry	0.82	18	0.21	0.53	1.45	0.82	10	0.13	0.69	1.03	0.08	0.93
Vertical symmetry	1.04	18	0.09	0.9	1.24	0.97	10	0.07	0.9	1.07	2.3	0.03
No of indents	28.83	18	9.37	6	42	9.8	10	3.99	4	15	6.07	2.00E-06
Indent depth mean	11.18	18	1.47	1.25	2.26	9.32	10	1.41	0.57	0.9	11.04	2.58E-11
Indent width mean	1.76	18	0.3	8.4	13.6	0.68	10	0.09	6.9	10.7	3.25	0.003

4.3.3 Functional trait variation between *B. plebeja* and *B. conchifolia*

To assess functional and resource use trait variation between the species net assimilation rate, stomatal conductance, chlorophyll content, total nitrogen, total carbon, leaf mass area and leaf dry matter content were measured / calculated. Based on the normality criteria, the significance of differences for these traits was tested either using student's t-test or Kolmogorov–Smirnov test (K–S test) in the PAST software. The results of the tests are discussed in the section below and illustrated in Table 4.6.

❖ **Net assimilation rate**

The first step in obtaining the net assimilation rates for both the species was the generation of light response curves. Light response curves describe the photosynthetic utilization of light quantitatively. The curves are distinguished by cardinal points such as light compensation and saturation points. Comparisons of light response curves and their cardinal points provided distinctive characters for *B. plebeja* and *B. conchifolia*. To generate the light response curves, a single sample for *B. plebeja*, *B. conchifolia* and one of the mapping population plants (B08-360-46) between both the species was used. The main cardinal point of interest was the light saturating points i.e. the point at which light availability is no longer limiting to the photosynthetic process.

Light Response curves

In darkness (zero PAR), the net assimilation rates were zero for *B. plebeja*, *B. conchifolia* and the F1BC1 plant from the mapping population. Net CO_2 release gradually reduced and the CO_2 uptake of leaves increased gradually with increasing radiation. The CO_2 uptake then gradually levelled off before it reached its maximum value. Figure 4.1, represents key cardinal points for the species and the F1BC1 plant.

B. plebeja, was better able to use strong light and has a high light saturation point (800 – 1000 µmol m^{-2} s^{-1}). The considerably larger photosynthetic yield could be due to the greater capacity of the electron transport system and higher carboxyalase activity or to anatomical differences. *B. conchifolia* leaves use weak light better than *B. plebeja* leaves and reach their light saturation point at relatively low irradiance level (400 µmol m^{-2} s^{-1}). The plant from the mapping population (B08-360-48) had a higher light saturation point (700 – 900 µmol m^{-2} s^{-1}) and a higher net assimilation rate then *B. plebeja* and *B. conchifolia*, suggesting hybrid vigour.

Since the light saturation points varied for both the species and the F1BC1 hybrid it was decided to use an average of all the saturation points. A saturation point of 700 µmol m^{-2} s^{-1} was used to measure the net assimilation rate for the rest of the Gireoudia sample and the plants in the mapping population (Table 4.3). The net assimilation rate values in chapter 3 for the species in section Gireoudia were measured at this light level.

Table 4.3: Differences in light response of net photosynthesis of single leaves of *B. plebeja, B.conchifolia* and F1BC1 plant from the mapping population. Light curves were generated under stable supply of CO_2 concentration at 380 ppm and a light intensity of 700 µmol m^{-2} s^{-1}. The optimal temperature ranged from 21-23 °C.

cardinal point	*B. plebeja*	*B. conchifolia*	*F1BC1 Plant*
Respiration Rate	-0.26	-0.27	-0.31
Light saturation point	700	300	700
Net Assimilation Rate	4.05	0.94	4.78

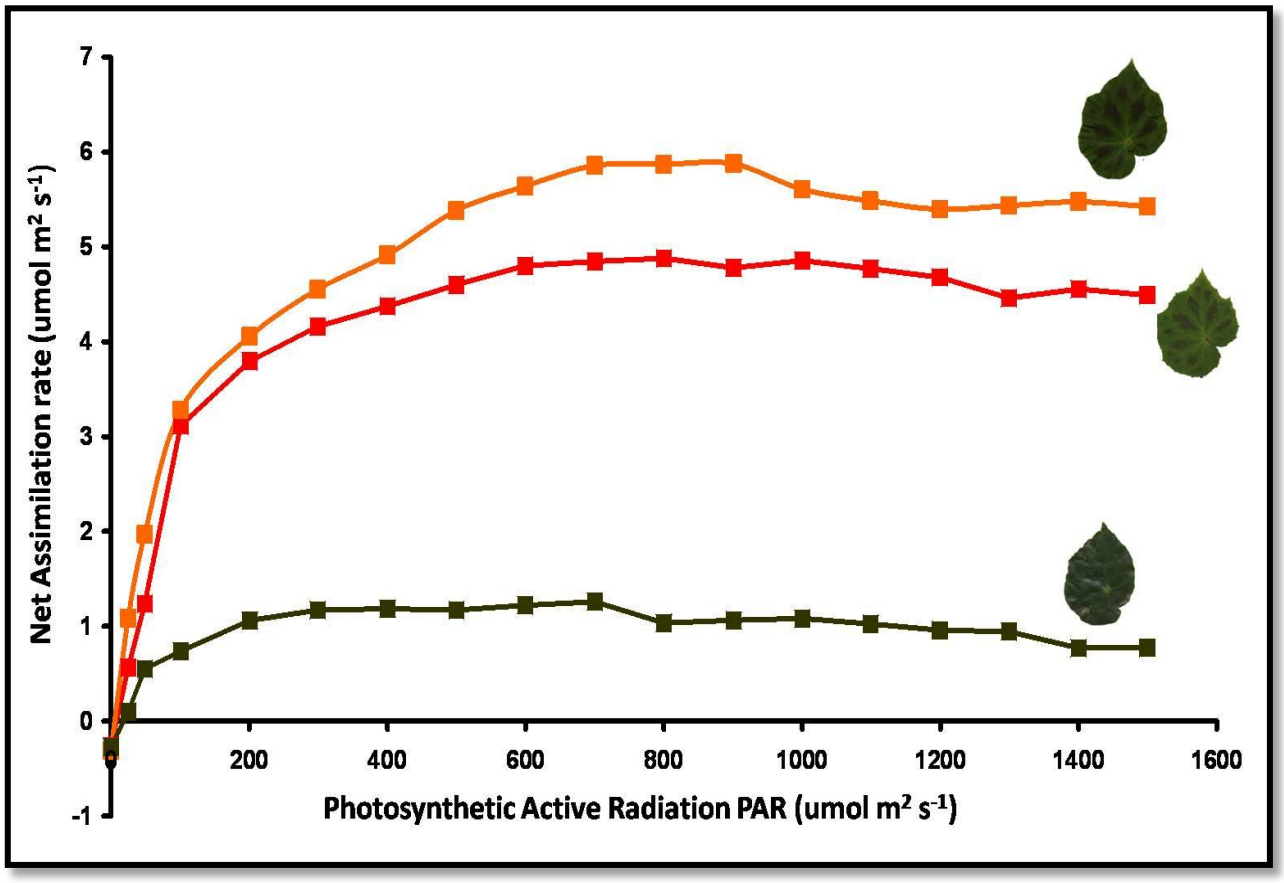

Figure 4.1: Differences in light response of net photosynthesis. Single leaves of *B. plebeja* (----), *B.conchifolia* (----) and an F1BC1 plant from the mapping population (----) were taken and net photosynthesis was measured under a stable supply of CO_2 concentration at 380 ppm and a light intensity of 700 µmol m^{-2} s^{-1}. The optimal temperature ranged from 21-23°C.

Diurnal changes of CO_2 Net assimilation rate

The net assimilation rates of plants are always subject to seasonal and diurnal changes. The seasonal and diurnal changes are geared by the stage of shoot development, the age of the leaf, as well as the accumulation of hormones and of carbohydrates in the leaves (Larcher, 2003). Apart from these, changes in the environmental factors such as light intensity, leaf temperature, air temperature and vapour pressure deficit are also responsible for the diurnal changes in the net assimilation rate (Lakso, 1985; Downtown et al., 1987; Flore and Sams, 1986). In the light of this diurnal curve of CO_2 net assimilation rates were generated for *B. plebeja*, *B. conchifolia* and B08-360 in order to get an idea of the peak photosynthetic time of the day. To further broaden our investigation it was decided to evaluate diurnal changes for stomatal conductance in *B. plebeja*, *B. conchifolia* and B08360 (Figure 4.2).

Diurnal curves for *B. plebeja* revealed that the rates of leaf gas exchange were the highest at the start of the day i.e. from six to eleven in the morning, followed by a decline in net carbon assimilation. By dusk, the net CO_2 exchange is reduced to 1.4 µmol m^{-2} s^{-1}.

Similar to *B. plebeja*, *B. conchifolia* photosynthesis was also highest at the start of the day i.e., from seven to eight o'clock and then experienced a gradual decline in net carbon assimilation in the following hours. After six in the evening CO_2 exchange was not longer at measurable rates and CO_2 release takes place. The F1 hybrid between *B. plebeja* and *B. conchifolia* also had high rates of leaf gas exchange at the start of the day, but by dusk, the net CO_2 exchange is reduced to 1.03 µmol m^{-2} s^{-1}.

Diurnal changes of stomatal conductance rate

Diurnal curves for *B. plebeja* revealed that the rates of stomatal conductance were the highest at the start of the day. The stomatal conductance was also lowest at about dawn and dusk,

with values of 31.7 mmol m^{-2} s^{-1} and 16 mmol m^{-2} s^{-1} respectively. Its maximum value was 63 mmol m^{-2} s^{-1} at about seven to eight o' clock in the morning. A similar pattern was also observed in the *B. conchifolia* leaf, where leaf conductance was highest at the start of the day i.e., from seven to eight o'clock and then experienced a gradual decline in the following hours. The stomatal conductance at about dawn was 17.3 mmol m^{-2} s^{-1} and 10.1 mmol m^{-2} s^{-1} for *B. conchifolia*. Its maximum value was 65 mmol m^{-2} s^{-1} at about seven to eight o' clock in the morning. The F1 hybrid between *B. plebeja* and *B. conchifolia* also had high rates of stomatal conductance rates at the start of the day. The stomatal conductance was 43.4 mmol m^2 s^{-1} at about dawn and 31.1 mmol m^2 s^{-1} at dusk. Its maximum value was 62.8 mmol m^{-2} s^{-1} at about seven to eight o'clock in the morning.

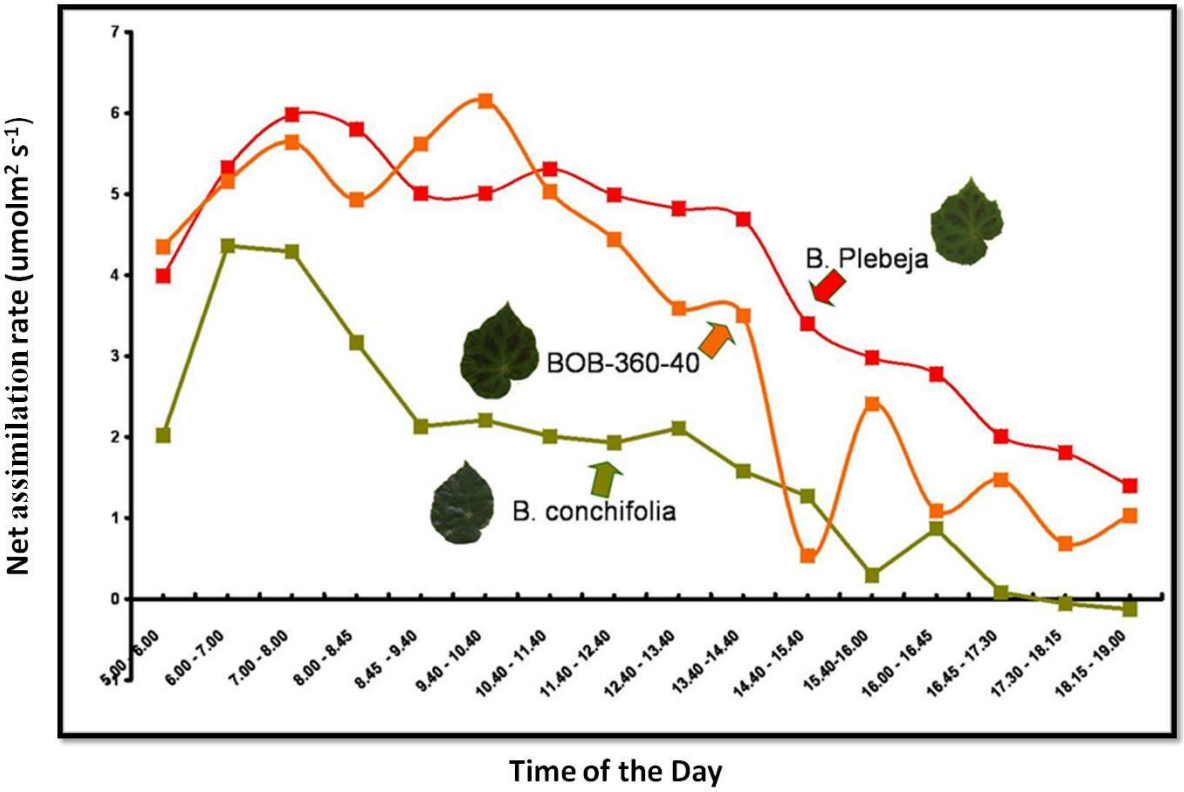

Figure 4.2: Differences in diurnal response of net photosynthesis from dawn to dusk. Single leaves of *B. plebeja*, *B.conchifolia* and BOB-360-40 plant from mapping population were taken and net photosynthesis was measured under conditions of ambient CO_2 and optimal temperature using a LICOR 6400.

Significance of differences between *B. plebeja* and *B. conchifolia*

Significance of differences for these traits was tested using student's t-test or Kolmogorov–Smirnov test (K–S test) in the PAST software. The results of the tests are discussed in the section below and illustrated in Table 4.4.

- **CO_2 Net Assimilation rate**

B. plebeja has a slightly higher net assimilation rate of 2.95 umolm^2s^{-1} (n= 19, sdev= 2.29) compared to *B. conchifolia* that has a mean of 1.49 umolm^2 s^{-1} (n= 7, sdev = 0.60), but this difference is not significant at the 5% level

- **Stomatal conductance**

B. plebeja has slightly higher stomatal conductance value of 70.9 mmol m^2s^{-1} (n= 19, sdev = 62.0) compared to *B. conchifolia* that has a mean of 41.2 mmol m^2s^{-1} (n= 7, sdev = 12.4), but this difference is not significant at the 5% level

- **Chlorophyll content**

B. plebeja has a slightly lower chlorophyll content of 38.1 spad (n= 21, sdev = 8.67) compared to *B. conchifolia* that has a mean of 42.0 spad (n= 14, sdev = 10.4), but this difference is not significant at the 5% level

- **Leaf nitrogen**

B. plebeja has significantly higher leaf nitrogen content of 4.19 percent (n= 11, sdev = 1.19) compared to *B. conchifolia* that has a nitrogen content of. 2.56 percent (n= 6, sdev = 1.05) ($P < 0.05$). .

❖ **Leaf carbon**

B. plebeja has slightly higher leaf carbon content of 49.0 percent (n= 11, sdev = 6.36) compared to *B. conchifolia* that has a nitrogen content of 45.7 percent (n= 6, sdev = 6.09). This is not significant at the 5% level.

❖ **Leaf mass area**

Significant variation between the species exists for leaf mass area content ($P < 0.05$). *B. plebeja* has significantly lower leaf mass area of 21.23 g m^{-2} (n= 12, sdev = 4.08) than *B. conchifolia* with a leaf mass area content of 44.9 g m^{-2} (n= 6, sdev = 14.16).

❖ **Leaf dry matter content**

B. plebeja has slightly lower dry matter of 48.68 mg g^{-1} (n= 12, sdev = 2.35) compared to *B. conchifolia* that has a high dry matter of 50 mg g^{-1} (n= 6, sdev = 12.39). This is not significant at the 5% level.

Table 4.4: Functional and resource use traits between *B. plebeja* and *B. conchifolia*. Mean , samples size (n), t/D and probability value (P) for net assimilation rate (umol m^{-2} s^{-1}), stomatal conductance (mmol m^{-2} s^{-1}), chlorophyll content (spad), nitrogen content (%), carbon content (%), leaf mass area (g m^{-2}) and leaf dry matter content (mg g^{-1}) are given. Significance of differences for net assimilation rate and stomatal conductance were tested using t test. The rest of traits were measured with KS test.

Trait	*B. plebeja*				*B. conchifolia*					
	Mean	**n**	**SD**	**Range**	**Mean**	**n**	**SD**	**Range**	**t */D**	**P**
Net assimilation rate	3.03	19	2.29	0.1 - 8.5	1.77	7	0.6	0.41-2.12	1.41	0.17*
Stomatal conductance	70.9	19	62	10.1 - 290	41.2	7	12.4	25.7 - 61.3	1.24	0.22*
Chlorophyll content	38.1	21	8.67	19.7 - 51.0	42	14	10.4	20.6 - 60.93	0.41	0.38
Nitrogen content	4.19	11	1.19	2.3 - 6.2	2.56	6	1.05	1.4 - 2.8	0.65	0.04
Carbon content	49	11	6.36	35.7 - 58.8	45.7	6	6.09	39.8 - 55.7	0.40	0.42
Leaf mass Area	21.23	12	4.08	28.3 - 65.3	44.91	6	14.16	28.3 - 65.3	0.91	<0.001
Leaf dry matter content	48.68	12	2.35	44.4 - 52.0	50	6	12.39	38.4 – 71.1	0.5	0.18

4.3.4 Leaf anatomical traits

Leaves in *Begonia* section Gireoudia have their mesophyll concentrated to form a narrow band in the centre of the leaf. The mesophyll is surrounded by an envelope of water cells (hypodermis) that in turn are bounded by the upper and lower epidermis. The presence of the hypodermis is a distinguishing feature of *Begonia* anatomy and may provide a cheap method of support for the leaf in a habitat where light for carbon fixation may be more limiting than the water. These water cells are also seen in other groups such as *Pepperonium* (Horner, 2012).

B. plebeja leaves are characterised by elliptical epidermal cells whereas *B. conchifolia* leaves are characterised by flat epidermal cells. The hypodermal attributes also vary between the species. The hypodermis of *B. plebeja* comprises columnar pallisade cells while *B. conchifolia* hypodermis is characterised by many layers of cells (Figure 4.3B).

The water tissue above and below the mesophyll consists of thin walled cells which vary somewhat in size and shape. In the case of *B. plebeja* the adaxial hypodermal cells are elliptical polyhedral cuboidal whereas the abaxial cells are polyhedral cuboidal where as in case of *B. plebeja* and *B. conchifolia* abaxial and adaxial cells are classified as polyhedral. The length and width of the adaxial and abaxial hypodermal cells also vary for both the species (Figure 4.3B).

The mesophyll is divided into pallisade and spongy layers. *B. conchifolia* has a much deeper spongy mesophyll that might be scattering light to increase the chances of light absorption (Figure 4.3B).

Stomatal traits vary between both the species. Both the species have single and clustered stomata. *B. plebeja* has a large number of stomatal clusters per mm^2 of leaf. *B. plebeja* clusters are also bigger and have significantly higher numbers of stomata in each cluster.

Another epidermal feature that varies between the species is the presence of trichomes on the leaf. Both the species possess whiplash and glandular trichomes on their abaxial and adaxial sides of the leaf. However the species vary in the distribution of whiplash and glandular trichomes (Figure 4.3C).

B. plebeja leaves have veins which are well developed and protruding, whereas those of *B. conchifolia* are thinner and embedded in the leaf. The veins emerge from petioles with a well developed vascular system consisting of an inner ring and an outer ring of vascular bundles. The vascular bundles are more or less oval shaped in cross section. In addition to the two concentric rings a central vascular bundle is present in *B. plebeja. B. plebeja* leaf is supported by a slightly higher number of bundles that are also larger compared to those of *B. conchifolia* (Figure 4.3D).

To investigate the significance of anatomical differences between *B. plebeja* and *B. conchifolia* a two sample t test was carried out. The results confirmed significant differences for a few of the anatomical traits such as width of lamina, width of abaxial hypodermis, width of mesophyll, depth of pallisade mesophyll and depth of spongy mesophyll (Table 4.5 - Table 4.7).

Figure 4.3: Characteristic differences between *B. plebeja* and *B. conchifolia* plants adapted to sunny *v.* shady extremes. (A) Differences in overall plant architecture of both the species; *B. plebeja* is characterised by large and thin leaf whereas *B. conchifolia* is characterised by small and thick leaf. (B) Differences in internal leaf anatomy; *B. plebeja* is characterised by thin leaf with smaller spongy mesophyll area and vice versa (C) Differences in trichome related attributes; *B. plebeja* is characterised by thin leaf with larger mesophyll area and vice versa (D) Differences in vascularisation attributes; *B. plebeja* is characterised by thin leaf with larger mesophyll area and vice versa.

Table 4.5: Traits related to leaf anatomy between *B. plebeja* and *B. conchifolia*. Mean, samples size (n), t and probability value (P) for anatomical attributes are also given. The unit of measurements for the traits is mm.

Trait	*B. plebeja*				*B. conchifolia*					
	Mean	**n**	**SD**	**Range**	**Mean**	**n**	**SD**	**Range**	**D**	**P**
Depth of lamina	0.42	15	0.21	0.15 - 0.75	0.70	15	0.15	0.45 - 0.92	0.60	**<0.001**
Depth at vein	0.42	5	0.22	0.18 - 0.76	0.67	4	0.05	0.59 - 0.72	0.60	0.21
Width of adaxial epidermis	0.05	15	0.09	0.01 - 0.39	0.04	15	0.01	0.02 - 0.05	0.53	**0.02**
Width at adaxial hypodermis	0.20	15	0.13	0.05 - 0.43	0.27	15	0.10	0.02 - 0.44	0.53	**0.02**
Width of adaxial cell	0.08	15	0.04	0.02 - 0.18	0.08	15	0.02	0.05 - 0.13	0.27	0.59
Width of abaxial epidermis	0.03	15	0.01	0.01 - 0.06	0.03	15	0.009	0.01 - 0.04	0.20	0.89
Width at abaxial hypodermis	0.07	15	0.03	0.04 - 0.01	0.20	15	0.03	0.15 - 0.27	1.00	**<0.001**
Width of abaxial cell	0.07	15	0.03	0.03 - 0.16	0.08	15	0.02	0.03 - 0.11	0.33	0.31
Width of mesophyll	0.09	15	0.02	0.03 - 0.13	0.15	15	0.03	0.11 - 0.21	0.80	**<0.001**
Depth of pallisade mesophyll	0.03	15	0.01	0.01 - 0.05	0.04	15	0.006	0.03 - 0.05	0.40	0.14
Depth of spongy mesophyll	0.05	15	0.02	0.02 - 0.09	0.10	15	0.02	0.07 - 0.15	0.93	**<0.001**

Table 4.6: Illustrate significance of differences for traits related to stomatal clustering and vascularisation patterns between *B. Plebeja* and *B. conchifolia*. Mean, samples size (n), t and probability values (*P*) for micromorphological attributes are also given. Lengths, widths and distance between clusters were measured in mm.

Trait	*B. plebeja*				*B. conchifolia*					
	Mean	**n**	**SD**	**Range**	**Mean**	**n**	**SD**	**Range**	**D**	**P**
No of stomatal clusters	31.4	9	12.1	16 - 45	26.4	9	5.7	18 - 34	0.44	0.25
Distance btw clusters	0.15	15	0.04	0.08 - 0.23	0.18	15	0.04	0.12 - 0.32	0.47	0.05
Stomatal cavity width	0.09	15	0.02	0.05 - 0.15	0.07	15	0.02	0.04 - 0.11	0.53	0.01
Stomatal cavity length	0.09	15	0.03	0.04 - 0.15	0.07	15	0.01	0.04 - 0.11	0.53	0.01
Stomata per cluster	3	15	0.92	1 - 4	1.93	15	0.59	1 - 3	0.60	<0.001
Petiole cross section width	2.26	4	0.63	1.75 - 3.12	2.2	4	0.6	1.6 - 2.92	0.25	0.99
Length of outer bundles	0.2	12	0.07	0.03 - 0.31	0.21	12	0.05	0.12 - 0.33	0.17	0.99
Width of outer bundles	0.13	12	0.05	0.08 - 0.23	0.11	12	0.04	0.07 - 0.18	0.25	0.78
Length of inner bundles	0.16	12	0.05	0.07 - 0.30	0.14	12	0.05	0.06 - 0.21	0.33	0.43
Width of inner bundles	0.11	12	0.02	0.07 - 0.15	0.07	12	0.03	0.03 - 1.31	0.75	<0.001

Table 4.7: Traits related to trichomes kind, density and length traits between *B. plebeja* and *B. conchifolia*. Illustrates mean, sample size (n), D and probability value (P) for traits related to trichomes kind, density and length. The unit of measurements used for measuring the length of trichomes is mm.

Trait	*B. plebeja*				*B. conchifolia*					
	Mean	**n**	**SD**	**Range**	**Mean**	**n**	**SD**	**Range**	**D**	**P**
Whiplash trichome length (Ab)	0.76	11	0.23	0.39 - 1.14	0.61	11	0.13	0.42 - 0.86	0.45	0.15
Whiplash trichome number (Ab)	19.7	11	16	2 - 44	41.2	11	32.8	7 - 102	0.45	0.15
Length of glandular trichomes (Ab)	0.16	6	0.31	0.03 - 0.81	0.05	8	0.005	0.04 - 0.06	0.50	0.25
Number of glandular trichomes (Ab)	0.54	11	0.82	0 - 2	3.90	11	7.16	0 - 24	0.36	0.37
Length of whiplash trichomes (Ad)	0.66	11	0.21	0.30 - 0.93	0.64	10	0.22	0.37 -1.14	0.36	0.37
Number of whiplash trichomes (Ad)	1.36	11	0.67	1 - 3	2.09	11	2.25	0 - 7	0.18	0.99
Length of glandular trichomes (Ad)	0.05	5	0.01	0.03 - 0.06	0.04	3	0.01	0.03 - 0.05	0.80	0.09
Number of glandular trichomes (Ad)	0.81	11	1. 07	0 - 3	0.09	11	.0 30	0 - 1	0.36	0.37

4.3.5 *B. plebeja* and *B. conchifolia* differences with special references to sun and shade plants

Comparisons of light and shade related differences drawn for *B. plebeja* and *B. conchifolia*, are discussed below (Table 4.8).

❖ **Leaf shape and area attributes**

Typical sun plant leaves possess significantly smaller surface area compared to leaves from typical shade plants. These small leaves are advantageous in dry environments owing to the fact that the smaller leaf will have a smaller boundary layer and better convective heat exchange with the environment. However in this study, *B. plebeja* has a significantly higher leaf area compared to *B. conchifolia*. This seems to be compensated by a more divided leaf area (high number of serrations) that would generate a thinner boundary layer and hence better convective heat exchange (Nobel, 1983; Schuepp, 1993).

❖ **Leaf function**

Plants that grow under high light intensities exhibit high net assimilation rates compared to shade plants under low light intensities. As expected, *B. plebeja* appears to have slightly higher photosynthetic rates compared to *B. conchifolia*. This is also coupled with a higher stomatal conductance for *B. plebeja* compared with *B. conchifolia*. Sun leaves typically contain a lower proportion of chlorophyll content compared to shade plants. This also holds true for these species.

❖ **Leaf anatomy**

An array of anatomical characteristics were looked at to see if the leaf anatomy of both the species complement what is seen for sun and shade plants in general.

(a) Trichomes and stomatal related traits

Sun leaves have generally more trichomes on the leaf surface to protect them from getting desiccated in sunny habitats. However in this study, *B. plebeja* does not have more whiplash and glandular trichomes compared to *B. conchifolia*. The survey also revealed that *B. plebeja* has slightly higher stomatal frequency. Another finding was that *B. plebeja* also possess a slightly higher number of stomata per cluster with each stoma having a small stoma size. Such traits are often associated with sunny habitats.

(b) Mesophyll

The general trend related to leaf thickness is that sun leaves are thicker than shade leaves with proportionally larger spongy and palisade mesophyll layers and vice versa. However *B. plebeja* leaf appears to be thinner then *B. conchifolia* and hence is not consistent with the general trend seen for sun plants. Clear differences in palisade and spongy mesophyll dimensions occur between both the species. The palisade to spongy mesophyll ratio is high in the case of *B. plebeja* (0.6) and low in case of *B. conchifolia* (0.4). This is consistent with pattern observed for sun and shade plants.

(c) Resource use traits

Another trait that differs between sun and shade species is leaf mass area; sunny plants usually have high leaf mass area compared to shade plants. However in our study *B. plebeja* (21.23 g m^{-2}) seems to have low leaf mass area compared to *B. conchifolia* (44.91 g m^{-2}).

Table 4.8: Characteristic differences between *B. plebeja* and *B. conchifolia* plants adapted to sunny *v.* shady extremes habitats.

Trait	*B. plebeja*	*B. conchifolia*
Leaf area	high	low
Thickness of leaf	low	high
Net assimilation rate	high	low
Light saturating point	high	low
Stomatal conductance	high	low
Chlorophyll content	low	high
Leaf mass area	low	high
Thickness of mesophyll	low	slightly high
Palisade spongy mesophyll ratio	high	low
Stomatal density	high	low
Stomatal per cluster	high	low
Leaf orientation	erect	horizontal

4.3.6 Conclusions

Apparent adaptive divergence is observed between the two studied species *B. plebeja* and *B. conchifolia*. Significant differences were observed between both the species for a number of phenotypic variables indicating that the two species are not only different in their habitat choice but have also undergone sufficient phenotypic divergence and display genetically based phenotypic differences in the controlled glass house conditions.

Documented evidence between *B. plebeja* and *B. conchifolia* revealed that many observations are consistent with the often generalised adaptive patterns of focusing and scattering light between light and shade plants. The leaf orientation incase of *B. plebeja* is erect i.e, it has leaves held vertically to expose less leaf area directly to the drying sun in the seasonally dry forests, while that of *B. conchifolia* is horizontal i.e, it holds leaf face flat or horizontally to the sunlight in order to capture light in the understory habitats. *B. plebeja* leaves are also characterised by elliptical epidermal cells that are geared for focusing direct light and generating the deeper focal points for even distribution throughout the leaves compared to *B. conchifolia* that is characterised by flat epidermal cells geared for providing much greater absorbing area for predominant levels of light in the shady understory. The hypodermis of *B. plebeja* comprises of columnar pallisade cells that propagate light deeper into the mesophyll, thus distributing light evenly. Compared to that *B. conchifolia* hypodermis is characterised by many layers of cells that are geared for the scattering of light within the leaf. Further to this, analysis of the deciduous *B. plebeja* and evergreen *B. conchifolia* contrast showed that *B. conchifolia* had considerably greater mesophyll tissue volume than *B. plebeja*. This could be a possible strategy to compensate the conductance for CO_2 with a larger volume of mesophyll. Considering the comparison between species *B. conchifolia* mesophyll has a much deeper spongy mesophyll that might be providing large quantities of air space to

generate large quantities of scattered light to increase light absorption by chloroplast within the chlorophyll.

Generalised adaptive patterns for trichome related traits that are involved in dissipating heat energy have also been observed between both the species. *B. plebeja* appears to have fewer but larger whiplash and glandular trichomes compared to *B. conchifolia*. The larger number of trichomes on *B. plebeja* leaves in seasonally dry forest would provide a better leaf surface covering and could have been involved in water economy in the seasonally dry forest conditions.

Significant variation for leaf size exists between both the species. *B. plebeja* has significantly bigger leaves with an average leaf area of 8848.6 mm^2 compared to *B. conchifolia* that produces small leaves with an average leaf area of 2081.6 mm^2. Leaf shape also differs between both the species. *B. conchifolia* leaf is much more circular compared to *B. conchifolia*. *B. plebeja* leaf has slightly higher number of indents then *B. conchifolia*. The indents in case of *B. plebeja* leaves are deep and wide whereas the indents in *B. conchifolia* leaves are shallow and narrow. Some studies have supported the idea that leaves with a short leaf lifespan (deciduous) and low leaf mass per area are more likely to be toothed (Peppe et al., 2011; Royer et al., 2005). Leaf teeth are also hypothesised to be involved in increasing the sap flow thereby delivering nutrients and other solutes to young, emerging leaves and hence increasing the photosynthesis and relative growth rate (Royer and Wilf, 2006). This could also hold true for *B. plebeja* and needs further investigation.

B. plebeja and *B. conchifolia* showed the typical sun-shade stomatal responses that have been reported in numerous other studies. Stomatal frequency is usually higher in plants that are exposed to full sunlight than in shade plants (Boardman, 1977; Bjorkman, 1981). The

absolute difference survey for *B. plebeja* and *B. conchifolia* revealed a slightly higher stomatal frequency for *B. plebeja.* However differences in stomatal frequency were not significant. Another pattern related to stomatal clustering attributes observed for sun and shade plants is a decline in *stomatal size* with decreasing irradiance (Cameron, 1970). The stomatal clusters incase of *B. plebeja* were bigger then *B. conchifolia* clusters. The bigger stomatal clusters incase of *B. plebeja* might have a role in the slightly higher net assimilation and transpiration rate.

At functional level the species also differ. The major difference is in the photosynthetic light utilization. *B. plebeja* is better able to utilize strong light in the seasonally dry forest. On the other hand *B. conchifolia* leaves are adapted to shade and saturates light at a much lower light intensities. Analysis of diurnal photosynthetic response curves for both the species and the individual from the mapping population showed asymmetric variation i.e, they showed the highest intensity of rate of leaf gas exchange in the first half of the day i.e. from six to eleven in the morning followed by a subsequent decline in net carbon assimilation in the proceeding hours. This is similar to patterns that are found in higher land plants. However incase of *B. conchifolia*, the photosynthetic CO_2 exchange did not takes place after six in the evening. Opposite to that CO_2 release takes place. This could be attributed to feedback effect of carbohydrate accumulation in the leaves rather than by a limiting effect of stomatal opening which is evident by the negative A_{max} values after 6 pm.

Despite the differences in light utilization and diurnal photosynthetic response of the species, non-significant differences exist for the rates of net assimilation rate and stomatal conductances between the species. Maximum rates of photosynthesis for *B. plebeja* ranged between 1.4 and 5.98 µmol m^{-2} s^{-1} and *B. conchifolia* ranged between 0.08 and 2.02 µmol m^{-2} s^{-1}. Net assimilation rate of both the species measured were not in complete agreement with

observations found for other herbaceous plants in the tropical forests. Herbaceous shade plants in the tropical forests on average have net assimilation rate values in the range of 5 µmol m^{-2} s^{-1} to 10 µmol m^{-2} s^{-1} where as those in dry habitats are marked by net assimilation rate values between 15 µmol m^{-2} s^{-1} to 30 umol m^{-2} s^{-1} (Larcher, 2003). However, interestingly both the species have net assimilation rate values below 5 µmol m^{-2} s^{-1}. The values could very well be true since all the species in section Gireoudia are well below this range. The non significant differences between both the species for net assimilation rates could be due to environmental factors such as temperature, relative humidity, soil moisture, and nutrient availability, all of which influence photosynthesis (Kozlowski et al., 1991).

Significant differences were found for resource use strategy traits such as nitrogen and leaf mass area. *B. plebeja* has characteristically higher nitrogen content and low leaf mass area and vice versa. The high nitrogen content incase of *B. plebeja* could largely be linked to slightly higher net assimilation rates since nitrogen is a part of photosynthetic machinery. The low leaf mass area suggests that *B. plebeja* invests low amount of carbon and nutrients in light-intercepting leaf. *B. plebeja* have leaves that are large and thin and require less force to tear apart or puncture and have a short life-span. On the other hand, *B. conchifolia* leaves have low nitrogen content and high leaf mass area. The high leaf mass area could be linked with long leaf life of *B. conchifolia* that essentially requires robustness. The lower nitrogen content could be linked to lower net assimilation rates and also might have been involved in imparting lower palatability to the long lived *B. conchifolia* leaves. The lower palatability could have been an advantage for *B. conchifolia* leaf to deter herbivores in tropical forests that are known to have high herbivory rates. *B. conchifolia* also possessed a greater life span compared to the deciduous *B. plebeja.* It is known that species with an inherently high LMA not only have a greater lifespan of leaves but also of roots (Ryser, 1996). This could provide *B. conchifolia* with a competitive advantage in its epiphytic habitat where nutrient supply

could be deficient by enhancing the residence time of nutrients in plants (Aerts and Chapin, 2000).

High LMA species *B. conchifolia* also have low relative growth rate and vice versa under glass house conditions compared to *B. plebeja* that has a fast relative growth rate and is deciduous. The large and thick rhizomes of *B. plebeja* support fewer numbers of leaves per cm^2 of its rhizome while much smaller and thicker leaves of *B. conchifolia* support large number of leaves per cm^2 of its rhizome. *B. plebeja* produces non peltate leaves that are thinner and bigger in size and deciduous in most of the populations while peltate leaves of *B. conchifolia* are smaller, thicker and succulent and is persistent in the populations.

This study investigated the hypothesis that leaf phenotypes between *B. plebeja* and *B. conchifolia* were genetically rather than environmentally determined. This chapter has provided enough support in evidence of this hypothesis. *B. plebeja* and *B. conchifolia* phenotypes varied for ecophysiological and morphological traits under same environmental conditions in the glasshouse. This also supports the hypothesis that species in section Gireoudia speciated through habitat adaptation to specific environments. Studying in detail the life history and functional traits for both the species also helped us determine that structural features that typify sun or shade plants also typify sun- or shade-grown *Begonia* species.

CHAPTER 5. Quantifying trait variation (QTL) in the mapping population

5.1 Introduction

Hybrid speciation may be one of the factors contributing to species diversity in large and diverse genera such as *Begonia*. Natural hybrids have been observed in the wild in this genus, however the frequency of their occurrence is very low (Twyford PhD thesis, 2012, Peng et al., 2009, Peng et al., 2000, Teo and Kiew 1999). Cross fertilization experiments have shown that F1 hybrids between related *Begonia* species can be generated in the glass house and supports the presence of weak reproductive isolating barriers (Tebbit 2005).

Niche divergence between parents and hybrids is an essential requirement for hybrid establishment (Templeton, 1981). A major question that has not yet been studied in *Begonia* is the achievement of niche divergence by the hybrids. This chapter explores whether morphological and ecophysiological traits exhibited by the mapping population, B08_360, is intermediate, parental-like, or extreme when compared to *B. plebeja* and *B. conchifolia*.

Trait values in the mapping population could either fall into a few distinct classes or a continuous range. The traits that fall into discrete classes are referred to as discontinuous traits. For example, the presence and absence of blotches on the adaxial side of the leaf is a discontinuous trait. It is fairly straightforward to test whether such a trait is controlled by a set number of mendelian loci using a chi-squared test against the expected ratios.

Many important traits such length and width of a leaf are quantitative in nature. These traits in a segregating population will often follow a bell-shaped curve. The bell shaped (or

normal) distribution can be generated by control from a large number of loci. Different types of gene effects e.g, additive, dominance, and epistatic effects can all contribute to the phenotype of a quantitative trait, but generally additive effects are the most commonly found ones (Rieseberg et al., 2003). Continuously distributed phenotypes cannot be analyzed in the same manner as discrete traits. Rather, these traits are defined with the help of statistical parameters such as mean and the variance. The normality of traits can be assessed by normality tests such as Shapiro-Wilk test or Kolmogorov-Smirnov test.

All traits are affected by the environment, some more than others. I have attempted to minimise this affect by growing the mapping population and its parents and the parental species on the same bench in the greenhouse, but due to lack of space for replicants environmental noise cannot be controlled for.

Examining the structure of trait variation in the mapping population would provide information on the genetics underlying these traits. The nature of the complexity of this novel genetic variation could be crucial to the process of evolution and adaptation (Lexer et al., 2003b). If the traits which allow species to thrive in a habitat are simple ones then the species could evolve very quickly and traits could easily spread between species after hybridisation. On the other hand, traits which are complex and are regulated by many loci would take longer to evolve and will not be easily transferred between species (Rieseberg et al., 1999).

As the mapping population is a backcross I expect that any dominant traits from *B. plebeja* would be uniformly *B. plebeja* phenotype in the mapping population. Traits recessive from *B. plebeja,* if regulated by a single locus, would result in a 1:1 ratio of *B. plebeja* to *B. conchifolia* phenotype. Dominant traits from *B. conchifolia* may result in a 3:1 phenotype in the mapping population if regulated by a single locus. Simple genetics such as these would

suggest that the traits are amenable to further genetic analysis, perhaps even mapping. I also expect to see traits which are transgressive, producing phenotypes outside the range seen in the parents. This reveals that a combination of positive and negative regulators exist in both parents which, when recombined in the mapping population, result in positive or negative shifts in the phenotype. Compared to the range of values seen for Gireoudia as a whole this can indicate the evolutionary potential of the trait in this section.

5.2 Objectives:

This chapter investigates the phenotypic distributions of morphological, ecophysiological and micromorphological traits in an F1BC1 mapping population, BO8-360, between *B. plebeja* and *B. conchifolia*. The trait variation has also been investigated with special reference to transgressive segregation.

This chapter addresses the following two hypotheses:

1. As the population is a back cross to *B. plebeja* I expect most traits to vary around the *B. plebeja* mean rather than the *B. conchifolia* mean. Traits which vary about the *B. conchifolia* mean would be dominant and this would increase the speed at which they could fix in a population.

2. I expect that traits which measure the output of many systems in the plant (such as A_{max}) are more likely to show transgressive segregation in the F1BC1 than traits which are regulated by a single system (such as presence of anthocyanin in particular tissues).

5.3 Results:

This section investigates the distributions of morphological traits in the mapping population and investigates the complexity of the genetic basis of these traits.

5.3.1 Variation for morphological traits in the mapping population:

The segregation data for qualitative traits reveals that traits such as rhizome colour, absence and presence of blotches and absence and presence of a red petiole base are under simple genetic control. Some of the trait variation that is quantitative in nature seems to have complex genetic inheritance patterns. This is evident by frequency distributions for the traits. The univariate statistics (mean, minimum, maximum, standard variation values) for the quantitative traits in the mapping population are presented in detail for each category of traits.

5.3.1.1. Categorical traits:

a. Presence and absence of blotches:

Out of the parents used in the crosses, *B. plebeja*, was blotched, whereas *B. conchifolia* is blotch-less. The F1 plants segregated into 1:1 ratio with half of the individuals that were blotched and the other half plain suggesting that *B. plebeja* might be polymorphic for this trait (Figure 5.1A). The F1 hybrid CKB-137.8 used to generate the backcross was blotched. Based on single gene inheritance, a 3:1 ratio, or 1:1 ratio was expected for the individuals in the mapping population. However, the BC1F1 segregation for this cross showed 1.9:1 (2blotched:1blotch-less) ratio that did not fit for goodness by $\chi 2$ method (Table 21). The deviation from the expected ratio could be explained by the sensitivity of anthocyanin related traits to many environmental factors such as light, temperature, or water availability (Guo et al., 2008).

b. Stipule colour:

B. plebeja is characterised by green stipules (coded=0) where as *B. conchifolia* is characterised by stipules that possess strong red colouration (coded = 4). The F1 hybrid family segregated into weak to medium red colouration for stipules suggesting a semi

dominant effect from *B. plebeja* alleles. The mapping population segregated for stipule colouration into five categories namely 0 (green or white), 1 (green to faint pink), 2 (faint pink, weak pink or light pink), 3 (pink), 4 (pink-red or red-pink), and 5 (red). 31.93% of the individuals in the mapping population possessed green stipules (*B. plebeja* like) whereas 20.16% possessed red stipules (*B. conchifolia* like). The remaining individuals segregated into four classes; 15.96%, green faint pink (code=1), 9.24% faint pink (code=2), 15.12% pink (code=3), 7.56% red (5) (Figure 5.1B). Since many environmental factors as well as the time of scoring could affect the level of anthocyanin production, the traits were simplified to two categories; red and green. The BC1F1 segregation for this cross showed a 1.08:1 ratio that is not significantly different from 1:1 (p=0.64) suggesting monogenic nature of this trait (Table 5.2).

c. Petiole colour:

B. plebeja have green petioles (1) whereas *B. conchifolia* petioles are red (3).Petiole colour was semi-dominant from *B. plebeja* in the F1. Colour ranged from green to strong red with most petioles producing some anthocyanin (3weak: 3 medium red: 2 strong red) (Figure 5.1C). The F1 hybrid ckb-137.8 used to generate the backcross had red petioles. The F1BC1 population was scored as either green (41) or red (78), giving a ratio of 1.9:1 is significantly different from the expected 1:1 of a single dominant mendelian gene inherited from *B. conchifolia* ($P < 0.001$) (Table 5.2).

d. Petiole attachment point colour:

The petiole attachment point in *B. plebeja* leaves is characterised by a strong red colour whereas that of *B. conchifolia* leaves is characterised by the presence of a green eye. Red petiole attachment point phenotypes are observed in a few botanic gardens accessions of *B. conchifolia* (such as RBGE 20091107) but it is not reported in the wild. All the individuals in

the F1 hybrid family possessed clearly red or green eyes at the petiole attachment point (3:3). This suggests that *B. plebeja* is heterozygous for a dominant allele conferring the red eye phenotype. The F1 hybrid CKB-137.8 used to generate the backcross had a red coloured eye.

A total of 99 individuals in the mapping population possessed a red eye (*B. plebeja* trait) although in this case red includes a range of phenotypes weaker than that seen in the *B. plebeja* parent or the F1 hybrids. On the other hand 20 individuals lack the red eye (*B. conchifolia*) (Figure 5.1D). The BC1F1 population segregated in a ratio of 2.2:1 red to green, The BC1F1 population was then expected to segregate in a 3:1 ratio for the cross based on the parents both being heterozygous for the *B. plebeja* red eye allele. The BC1F1 population segregated in a ratio of 4.9:1 that did not fit for the expected ratio (Table 5.2) (P=0.03).

e. Presence and absence of red petiole base:

The base of petioles in *B. plebeja* leaves are characterised by accumulation of anthocyanins which are absent in *B. conchifolia*. F1 hybrids segregated into red and green petiole bases with 3 red and 3 green (1:1). The F1 hybrid CKB-137.8 used to generate the backcross had a red petiole base. 91 of the individuals in the mapping population possess red petiole base (*B. plebeja* trait) while the remaining 28 lacked the red petiole base (*B. conchifolia* trait) (Figure 5.1E). The BC1F1 population segregated in a ratio of 3.2: 1 for the cross which fitted a 3:1 ratio expected for a cross between two heterozygotes at P = 0.71 ($\chi 2$ test) (Table 5.2). Therefore, it seems that the presence and absence of red petiole base is controlled by a single gene in *Begonia*.

f. Margin colour strength:

B. plebeja have clearly red margins whereas *B. conchifolia* leaves lack red colouration at the margins. All members in the F1 hybrids segregated into red and green margins with 3 red and

3 green (1:1). This suggests that *B. plebeja* is heterozygous for a dominant allele conferring the red margin phenotype. The F1 hybrid ckb-137.8 used to generate the backcross had red margins. The BC1F1 population segregated in a ratio of 2.2:1 red to green, although in this case red includes a range of phenotypes weaker than that seen in the *B. plebeja* parent or the F1 hybrids. Chi squared support showed that margin colour strength could be controlled by a single gene (P = 0.12) (Table 5.2).

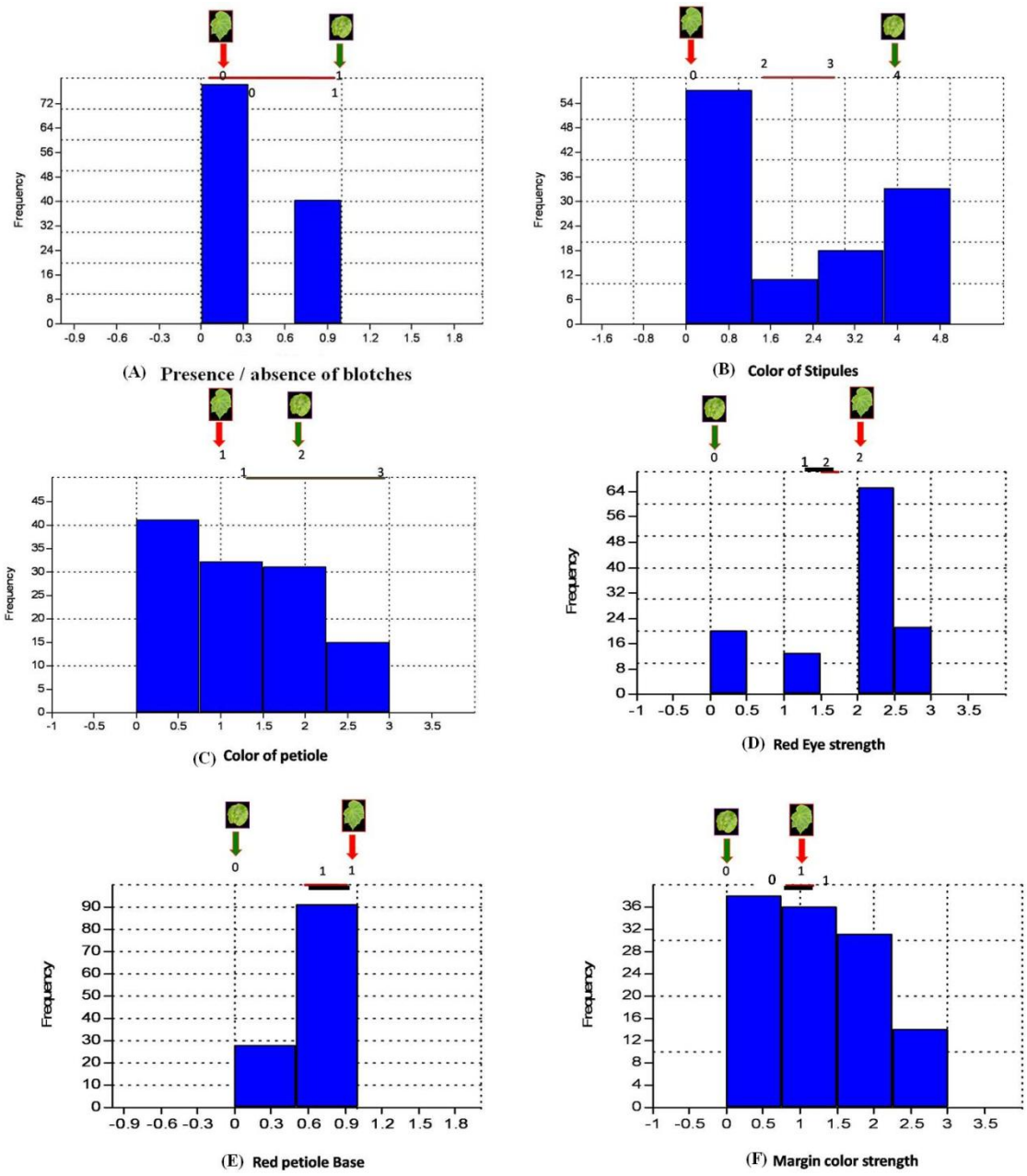

Figure 5.1: Histograms to illustrate categorical morphological traits including presence and absence of blotches (A), colour of stipules (B), colour of petiole (C) , Red eye strength (D), red petiole base (E) and margin colour strength (F). Arrows are used to point out values for *B. plebeja* marked as red and *B. conchifolia* marked as green as well as the range for F1 hybrid family marked as dashed line. A total of 119 individuals were used to generate these statistics.

Table 5.1: Illustrate segregation ratios for categorical morphological traits.

Plant	P1 × P2	F1 Phenotypes	F1BC1					
	(n = 2)	**(n = 6**	**1**	**2**	**3**	**4**	**5**	**6**
Colour of stipules	green × pink-red	faint pink : pink	green	pink	faint pink	pink red	green faint	red
		5:1	38	19	11	18	24	9
Colour of rhizomes	green × red	green : red	green	red				
		4:2	34	85				
Colour of petiole	weak red × strong	weak:mediumred:strong red	green	weak red	medium red	strong red		
		3:1:2	41	32	31	15		
Ab/pr blotches	present × absent	blotch : non-blotch	blotched	non				
		3:3	78	41				
Red eye strength	strong red × green	Red: green	Red	green				
		3:3	92	20				
Ab/pr petiole base	present × absent	present: absent	present	absent				
		3:3	28	91				
Margin colour strength	red × white/green	Green: red margins	green	weak red	red	dark red		
		3:3	31.9	30.2	26.0	11.7		

Table 5.2: Illustrate χ2 method for categorical morphological traits.

S. No.	Plant characters P1 × P2	F1 Phenotypes	F1BC1 observations	χ2 ratio	χ2 Value	P value
colour of stipules	green × pink-red	faint pink : pink	62 green : 57 red	1.08:1	0.210	**0.64**
colour of petiole	weak red × strong	weak:mediumred:strong	78 red : 41 green	1.9 : 1	11.5	0.0007
Ab/pr blotches	present × absent	Blotch : non-blotch	78 blotched : 41 Non blotched	1.9:1	5.67	0.01
Red eye strength	strong red × green	Strong red: green	99 red: 20 green	4.9:1	4.26	0.03
Ab/pr petiole base	present × absent	present : absent	91 present: 28 absent	3.2:1	0.137	**0.71**
margin colour strength	red × white/green	green : red margins	82 red : 37 green	2.2 : 1	2.35	**0.12**

5.3.1.2. Continuous morphological traits:

The frequency distributions data for continuous morphological traits revealed that most of the measured traits are determined by the combined effect of more than one pair of genes (Figure 5.2). The univariate statistics (mean, minimum, maximum, standard variation values) for the quantitative traits in the mapping population are presented in detail in Table 5.3.

The mapping population displayed variation for all the measured traits including length of stipules, total number of rhizomes, length of rhizomes, width of rhizomes, and total number of leaves produced (leaf + scars) per cm rhizome. The length of stipules ranged from 7 to 36 mm (Figure 5.2A), total number of rhizomes ranged from one to ten rhizomes per plant (Figure 5.2B), length of rhizomes ranged from 7.5 to 55 cm (Figure 5.2C), width of rhizomes ranged from 7.5 to 55 cm (Figure 5.2D), total number of leaves produced (leaf + scars) per cm rhizome ranged from 0.13 to 1.5 (Figure 5.2E) within the mapping population. The range of values observed is well beyond the parental values for most of the measured traits. This suggests a moderate to high transgressive inheritance in both negative and positive senses as the mapping population data display lower and higher values than the parents. The average frequency distribution for the mapping population and parents illustrated in Figure 5.2, show clearly this transgressive inheritance.

The frequency distributions data tested for normality with Shapiro-Wilk test further revealed that none of the trait showed a good fitting to normal distribution. The traits that were not normally distributed were further transformed for QTL analysis.

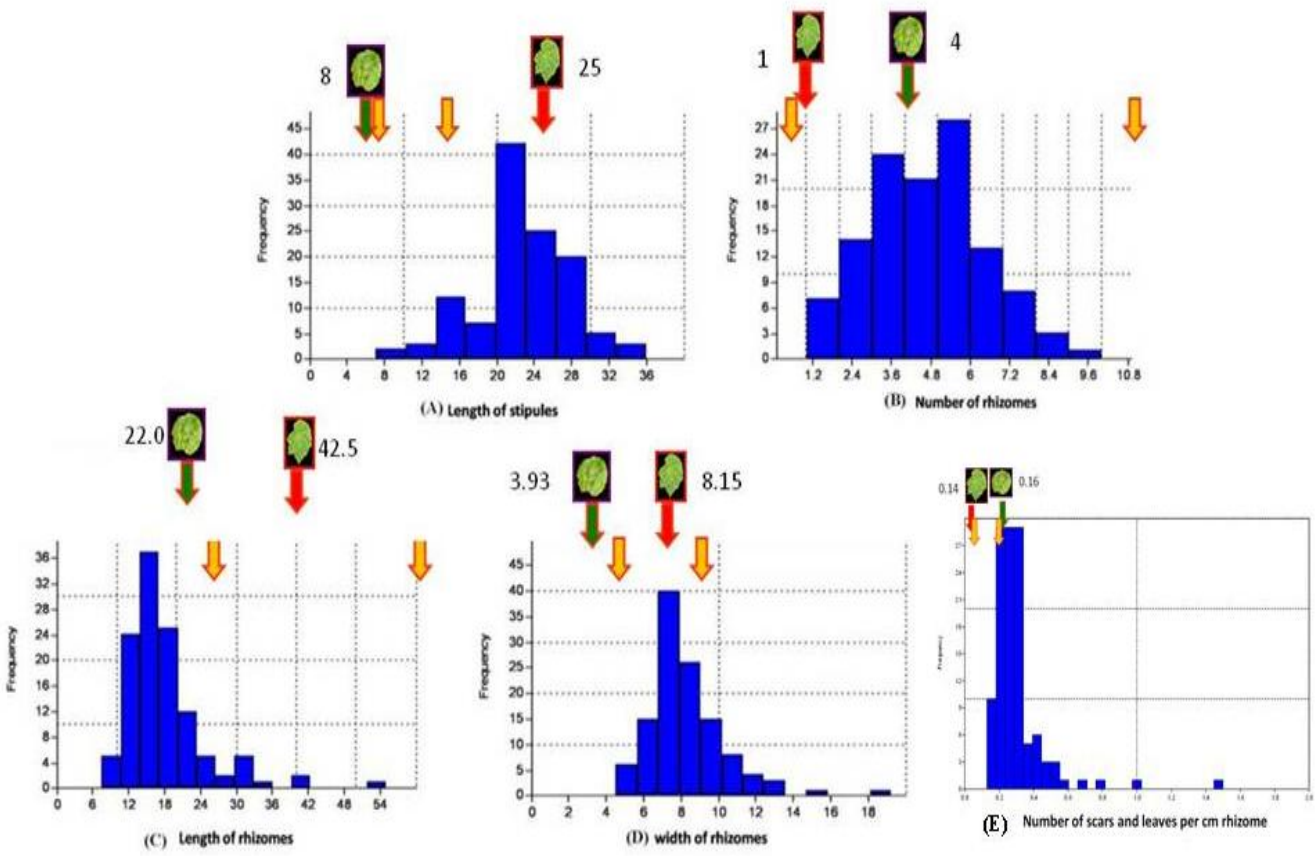

Figure 5.2: Illustrate histograms for quantitative morphological traits including length of stipules (A), number of rhizomes (B), length of rhizomes (C), width of rhizomes (D) and number of scars and leaves leaves produced per cm rhizomes (E). Arrows are used to point out values for *B. plebeja* marked as red and *B. conchifolia* marked as green as well as range for F1 hybrid family marked as dashed line. A total of 119 individuals were used to generate this statistic.

Table 5.3: Illustrate univariate statistics for the quantitative morphological traits measured for the mapping population. **The traits measured included stipule length (stipL), number of rhizomes (Nrhiz), number of scars (Nscr), length of rhizomes (Lrhiz), width of rhizomes (wrhiz), number of leaves (N.leaves), number of scars and leaves (sc&leaf) per cm rhizome. The length and width of different organs were measured in mm.**

	StipL	Nrhiz	Nscr	Lrhiz	wrhiz	Nleaves	sc&leaf/cm rhizome
N	119	119	119	119	119	119	119
Min	7	1	0	7.5	4.5	4	0.13
Max	36	10	6	55	19.23	35	1.5
Mean	22.57	4.19	0.34	17.97	8.29	15.85	36.11
Std. error	0.47	0.16	0.09	0.62	0.19	0.62	0.30
Variance	26.62	3.17	0.96	46.07	4.47	46.25	0.03
Stand. dev	5.16	1.78	0.98	6.79	2.11	6.80	0.17
Median	23	4	0	16.3	7.83	15	0.27
Skewness	-0.33	0.32	4.07	2.28	1.81	0.46	4.31
Shapiro-Wilk W	0.97	0.96	0.40	0.81	0.87	0.97	0.61
p(normal)	0.063	0.001	6.26E-20	5.95E-11	1.46E-08	0.034	2.70E-16

5.4 Variation for leaf size and shape attributes in mapping population:

The leaf size and shape trait variation that is quantitative in nature seems to have complex genetic inheritance patterns in *Begonia*. This is evident by frequency distributions for the traits (Figure 5.4, 5.5). The trait distributions data suggests that leaf size and shape attributes might be determined by the combined effect of more than one pair of genes. Several researchers agree that leaf size and shape traits are typical quantitative traits controlled by a complex genetic system (Tsukaya, 2005, Byrne, 2012; Pérez-Pérez et al., 2002; Feng et al., 2009). This is discussed in chapter 7 in detail. The univariate statistics (mean, minimum, maximum, standard variation values) for the quantitative traits in the mapping population are presented in detail in Table 5.4.

The mapping population displayed ample variation for all the measured traits. Variation for area of lamina ranged from 2214.2 mm to 21926.8 mm (Figure 5.4A), perimeter ranged from 168.4mm to 557.2 mm (Figure 5.4B), squared perimeter / area ranged from 10.95 to 26.74 (Figure 5.4C), circularity ranged from 71.65 to 94.81 (Figure 5.4D), width of leaf ranged from 48.6 – 149.1 mm (Figure 5.4E), length of leaf ranged from 62.35 – 219.4 mm (Figure 5.4F), length:width ratio ranged from 0.95–1.55 (Figure 5.5A), horizontal symmetry ranged from 0.26 –1.34 (Figure 5.5B), vertical symmetry ranged from 0.77–1.54 (Figure 5.5C), number of indents ranged from 11.5–39 (Figure 5.5D), indent depth mean ranged from 0.95–3.39 (Figure 5.5E), and indent width mean ranged from 8.87 – 18.11 (Figure 5.5F). The range of values observed is well beyond the parental values for most of the measured traits. This suggests a moderate to high transgressive inheritance in both negative and positive senses as the mapping population data display lower and higher values than the parents. The average frequency distribution for the mapping population and parents illustrated in the figures 5.4 and 5.5, show clearly this transgressive inheritance.

The frequency distributions data tested for normality with shapiro-Wilk test further revealed that seven out of twelve traits showed a good fitting to normal distribution. The five traits that were not normally distributed were further transformed for QTL analysis.

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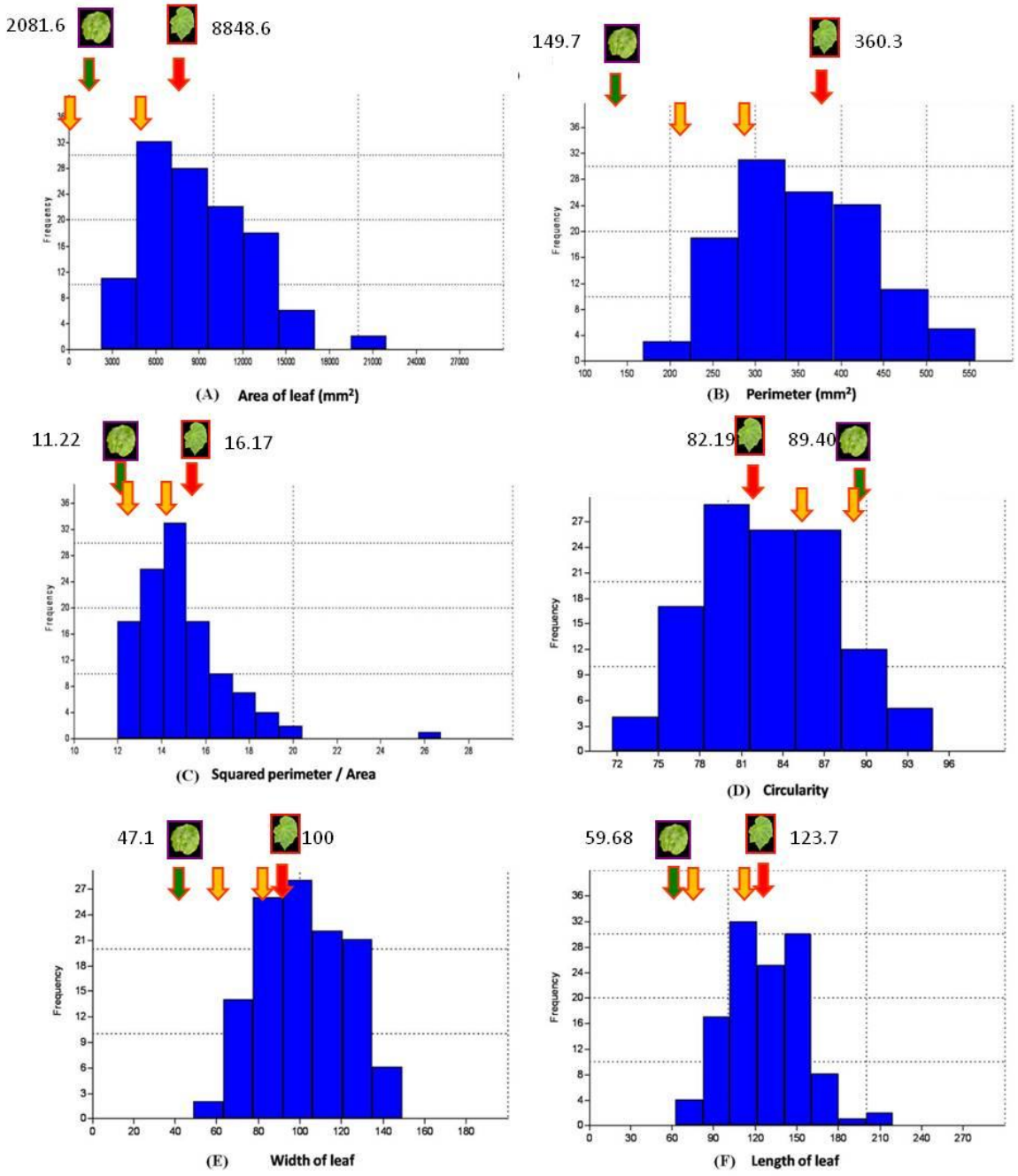

Figure 5.4: Histograms for area of leaf (A), perimeter (B), squared perimeter/Area (C), circularity index (D), width of leaf (E), and length of leaf (F). Arrows are used to point out values for *B. plebeja* marked as red and *B. conchifolia* marked as green as well as range for F1 hybrid family marked as dashed line. A total of 119 individuals were used to generate this statistic.

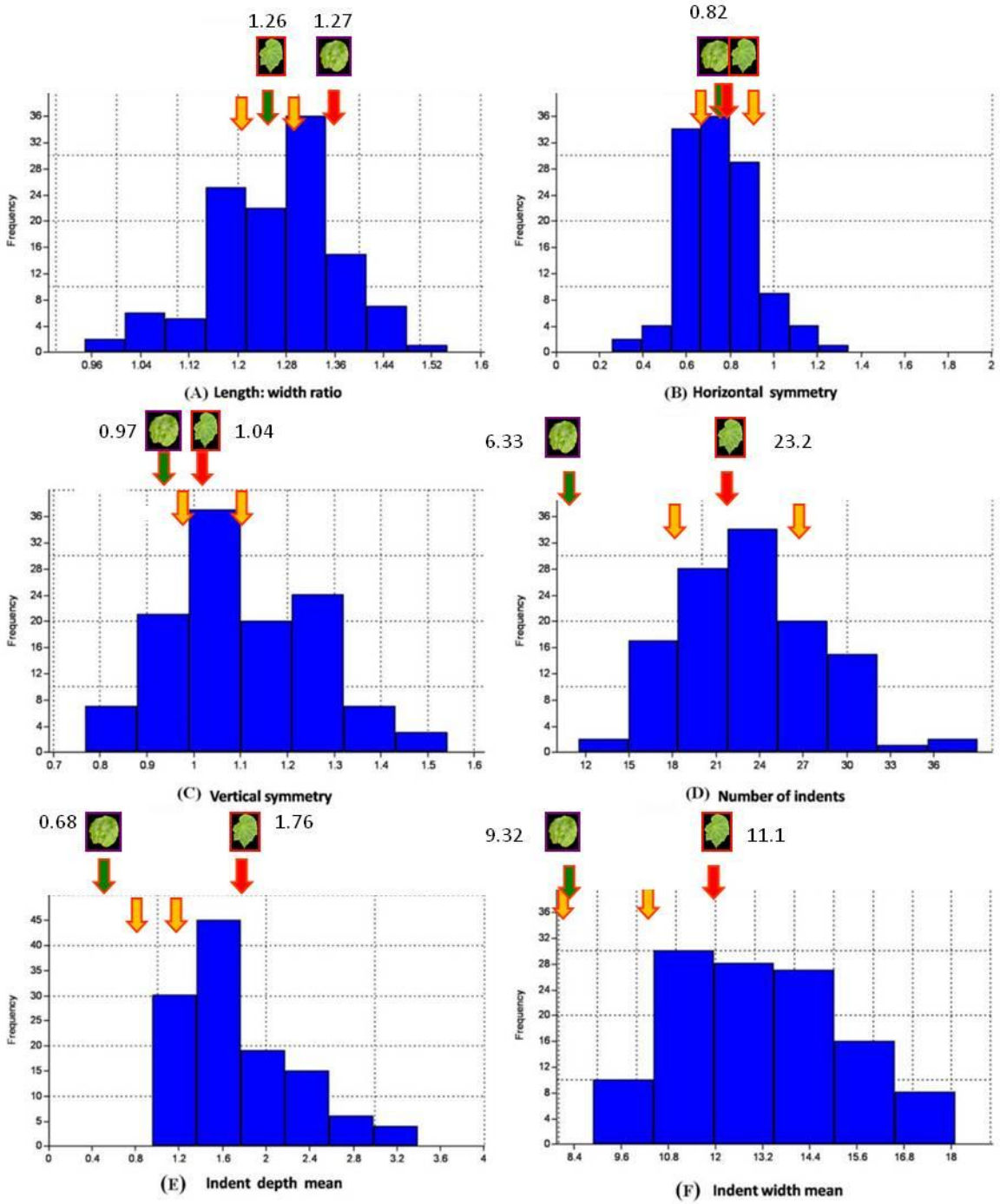

Figure 5.5: Histograms for length and width ratio (A), horizontal symmetry (B), vertical symmetry (C) , number of indents (D) , indent depth mean (E) and indent width mean (F). Arrows are used to point out values for *B. plebeja* marked as red and *B. conchifolia* marked as green as well as range for F1 hybrid family marked as dashed line. A total of 119 individuals were used to generate this statistic.

Table 5.4: Illustrate univariate statistics for the leaf size and shape attributes measured in the mapping population with the help of lamina software. The traits measured included Area(A_2), perimeter (P2), Squared perimeter/Area (SP2/A2), circularity index (C), width of lamina (w), Length of leaf (L), length and width ratio (l:W), horizontal symmetry (H.symm), vertical symmetry (ver.symm), number of indents (N.I), indent width mean (Iwm) and indent depth mean (Idm).

0	A2	P2	SP2/A2	C	w	L	l:W	H.symm	ver.symm	N.I	Iwm	Idm
N	119	119	119	119	119	119	119	119	119	119	119	119
Min	2214.25	168.46	10.9	71.65	48.6	62.3	0.95	0.26	0.77	11.5	8.870	0.95
Max	21926.80	557.27	26.7	94.81	149.1	219.4	1.55	1.34	1.54	39.0	18.110	3.39
Mean	9234.58	356.80	14.8	83.05	101.8	128.4	1.26	0.74	1.10	23.3	13.256	1.75
Std. error	352.50	7.25	0.19	0.45	1.95	2.56	0.01	0.01	0.01	0.4	0.191	0.05
Variance	14786900.0	6270.27	4.46	24.37	453.1	780.0	0.01	0.03	0.02	25.2	4.364	0.30
Stand.	3845.3	79.18	2.11	4.93	21.2	27.9	0.10	0.18	0.15	5.0	2.089	0.55
Median	8792.9	354.22	14.5	82.16	102.3	130.5	1.28	0.74	1.08	23.0	13.150	1.62
Skewness	0.75	0.24	1.90	0.18	0.07	0.27	-0.33	0.25	0.27	0.3	0.254	0.91
Shapiro-	0.95	0.98	0.87	0.98	0.98	0.98	0.98	0.98	0.97	0.9	0.9807	0.92
p(normal)	0.00055	0.37	1.78E-08	0.34	0.18	0.35	0.20	0.34	0.05	0.23	0.08	4.19E-06

5.5 Variation for leaf anatomical traits in the mapping population:

The segregation data for qualitative traits revealed that traits such as epidermal cell type and type of hypoderm cells are not under simple genetic control (Table 5.5). The trait distributions data for quantitative leaf anatomical traits revealed that these traits are determined by the combined effect of more than one pair of genes (Figure 5.6, 5.7 and 5.8). Researchers agree that there is little possibility that traits related to internal leaf anatomy are controlled by a single gene (Byrne, 2012; Tsuge et al., 1996; Kim et al., 1998; Tsukaya and Uchimiya, 1997). This is discussed in chapter 7 in detail.

The univariate statistics (mean, minimum, maximum, standard variation values) for the quantitative traits in the mapping population are presented in detail in Table 5.6. The mapping population displayed variation all the measured traits including depth of lamina that ranged from 0.18 to 0.69 mm (Figure 5.6A), depth at vein that ranged from 0.29 to 0.83 mm (Figure 5.6B), depth of adaxial epidermisthat ranged from 0.02 to 0.03 mm (Figure 5.6D), depth of adaxial hypodermis that ranged from 0.02 to 0.35 mm (Figure 5.6E), width of adaxial cell that ranged from 0.01 to 0.21mm (Figure 5.6F), number of adaxial hypodermis cell layers that ranged from 1 to 3 (Figure 5.7A), depth of abaxial epidermis that ranged from 0.01 to 0.05 mm (Figure 5.7E). depth of abaxial hypodermis that ranged from 0.04 to 0.15 mm (Figure 5.7F), width of abaxial cell that ranged from 0.01 to 0.17 mm (Figure 5.8A), number of abaxial hypoderm cell layers that ranged from of 1 to 2 (Figure 5.8B), depth of mesophyll that ranged from 0.07 to 0.20 mm (Figure 5.8C), depth of pallisade mesophyll that ranged from 0.02 to 0.09 mm (Figure 5.8D), depth of spongy mesophyll that ranged from 0.03 to 0.1mm (Figure 5.8E). The range of values observed is well beyond the parental values for most of the measured traits. This suggests a moderate to high transgressive inheritance in both negative and positive senses as the mapping population data display lower and higher

values than the parents. The average frequency distribution for the mapping population and parents illustrated in the Figures 5.6, 5.7 and 5.8, show clearly this transgressive inheritance. Further, seven out of twelve traits showed a good fitting to normal distribution. The five traits that were not normally distributed were transformed for QTL analysis.

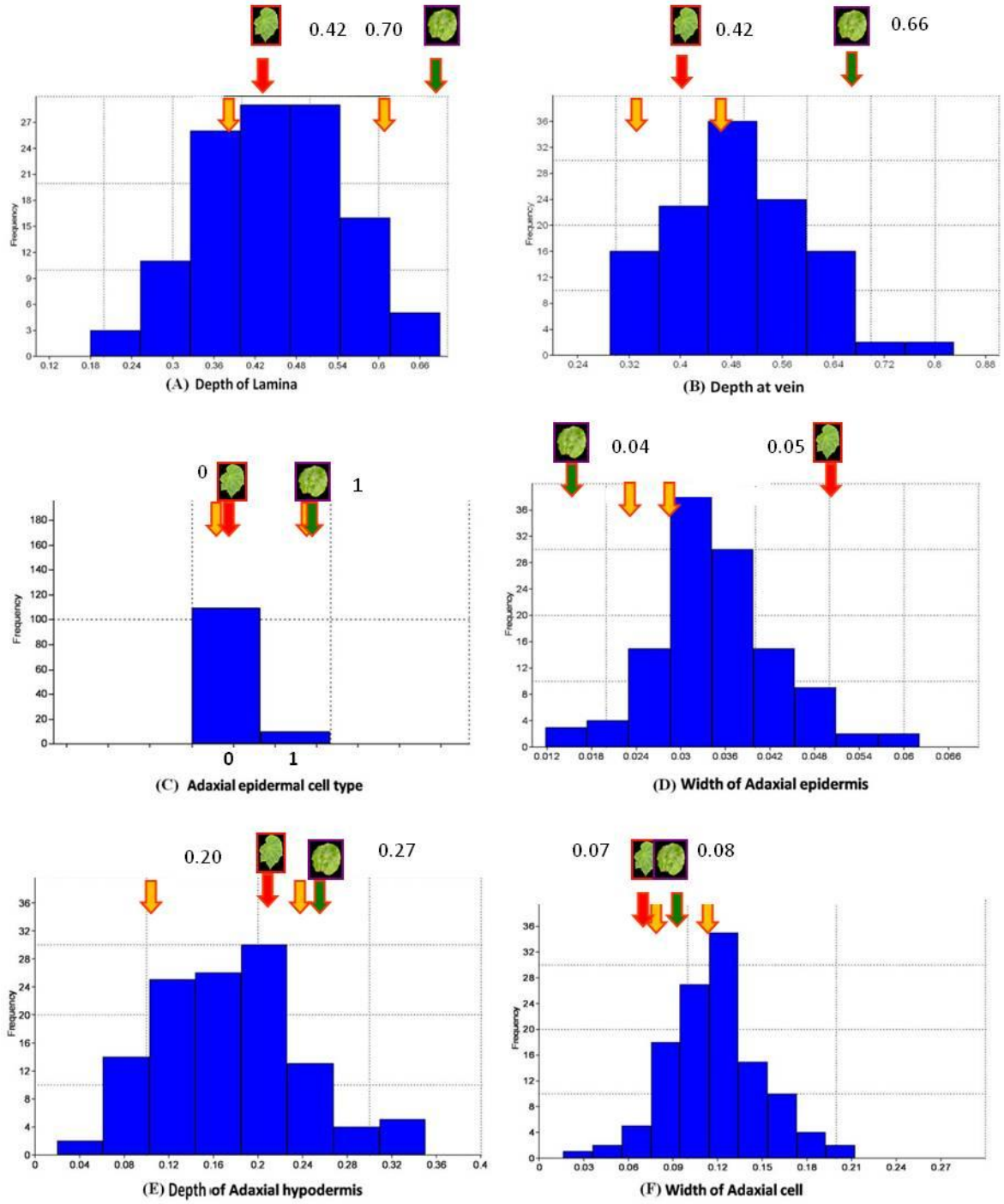

Figure 5.6: Histograms for the depth of lamina (A), depth at vein (B), adaxial epidermal cell type (C), width of adaxial epidermis (D), width of adaxial hypodermis (E) and width of Adaxial cell (F). Arrows are used to point out values for *B. plebeja* marked as red and *B. conchifolia* marked as green as well as range for F1 hybrid family marked as dashed line. A total of 119 individuals were used to generate this statistic.

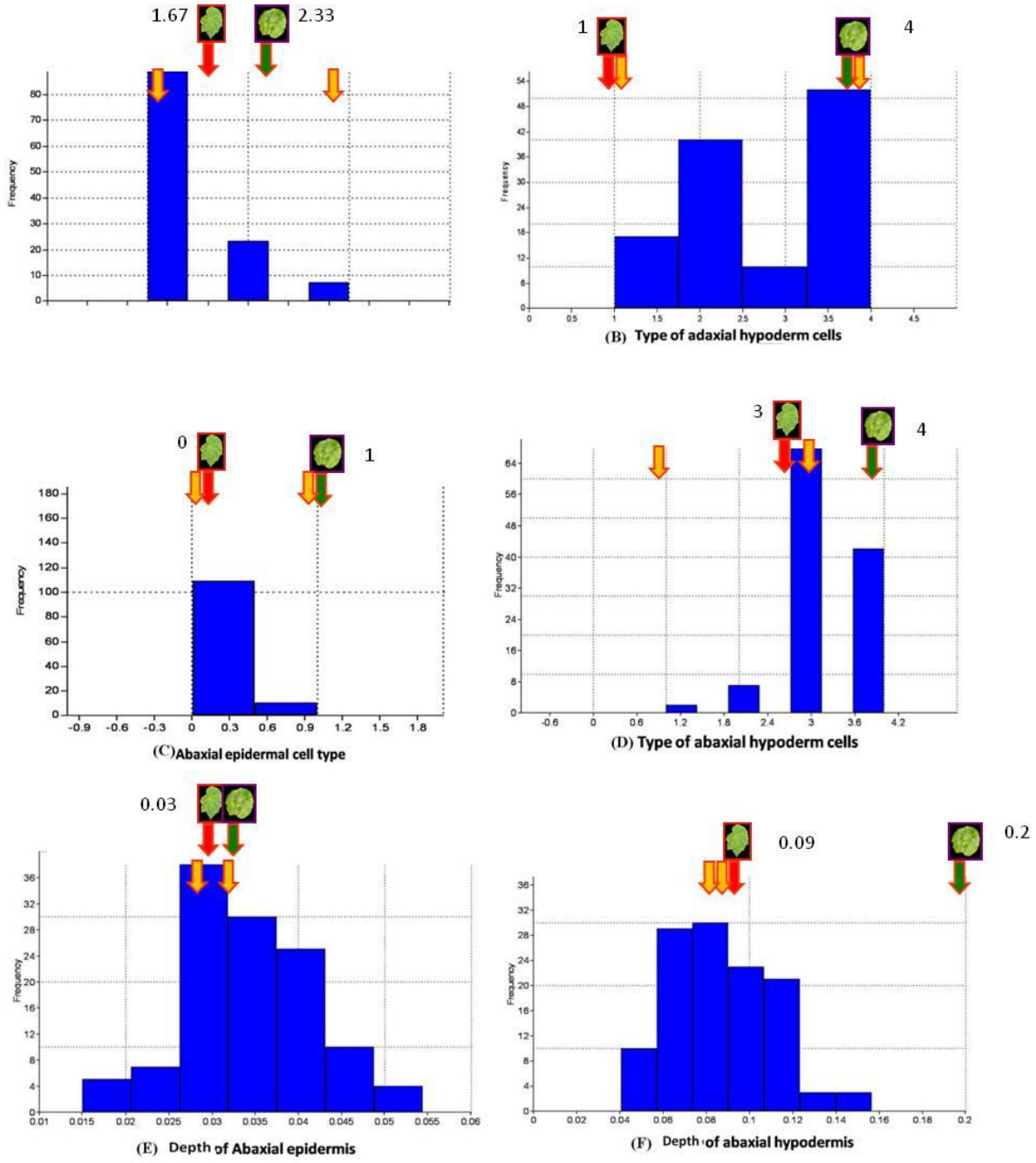

Figure 5.7: Histograms for the number of adaxial hypoderm cells (A), Type of adaxial hypoderm cells (B), abaxial epidermal cell type (C), type of abaxial hypoderm cells (D), width of abaxial epidermis (E), width of abaxial hypodermis (F). Arrows are used to point out values for *B. plebeja* marked as red and *B. conchifolia* marked as green as well as range for F1 hybrid family marked as dashed line. A total of 119 individuals were used to generate this statistic.

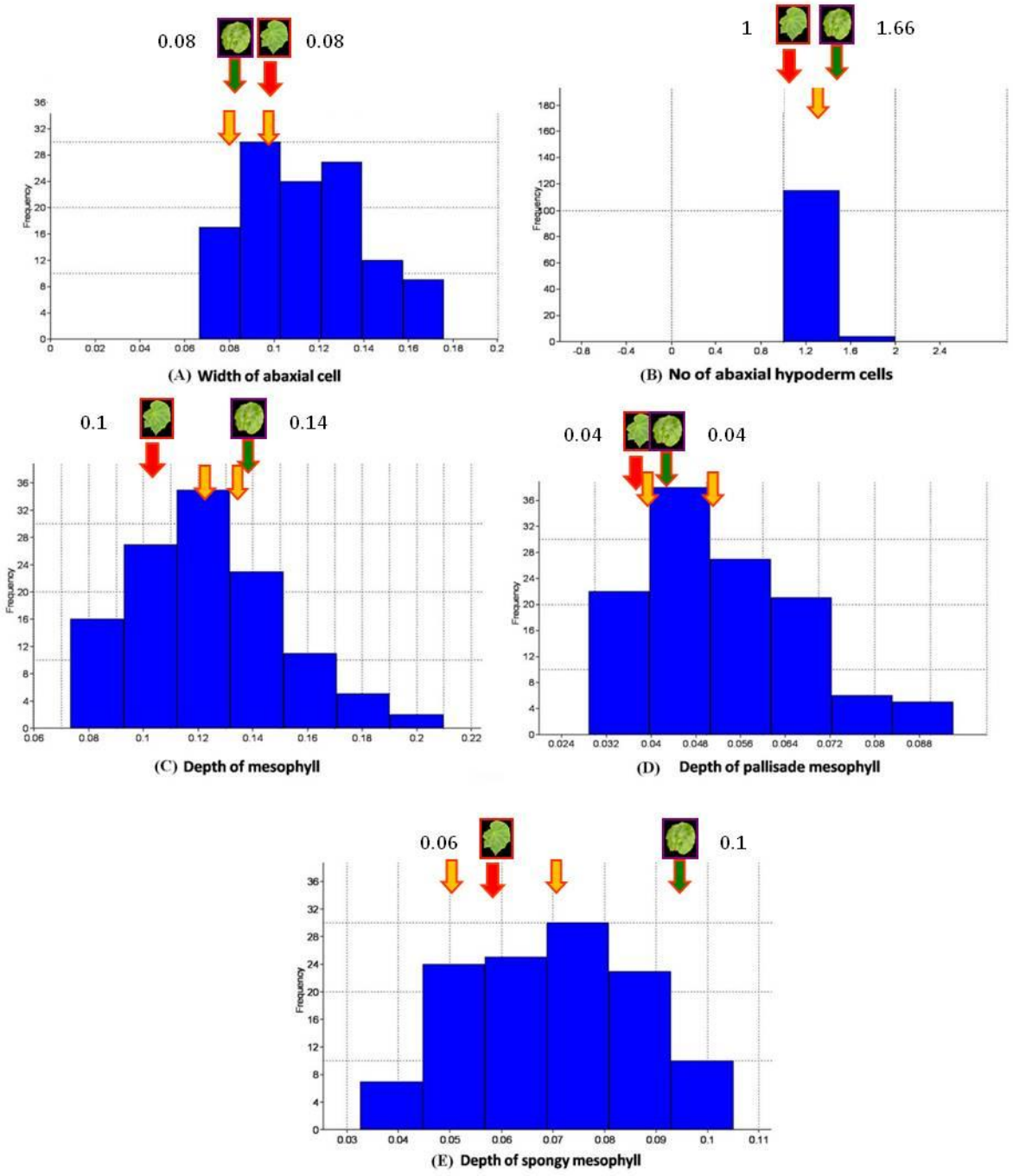

Figure 5.8: Histograms for the width of adaxial cell (A), number of abaxial hypoderm cells (B), depth of mesophyll (C), depth of pallisade mesophyll (D), and depth of spongy mesophyll (E). The depth and widths are measured in mm. Arrows are used to point out values for *B. plebeja* marked as red and *B. conchifolia* marked as green as well as range for F1 hybrid family marked as dashed line. A total of 119 individuals were used to generate this statistic.

Table 5.5: Segregation ratios for categorical micromorphological traits.

	P1 × P2 (n = 2)	F1 Phenotypes (n = 4)	F1BC1 observations			
			1	2	3	4
Adaxial epidermal cell type	elliptical × flat	elliptical - flat 1:1	elliptical 109	flat 10		
Abaxial epidermal cell type	elliptical × flat	elliptical - flat 1:1	elliptical 109	flat 10		
Type of adaxial hypoderm cells	elliptical polyhedral cuboidal × Polyhedral	elliptical polyhedral cuboidal × Polyhedral cuboidal 2:2	elliptical polyhedral cuboidal 17	polyhedral cuboidal 40	polyhedral spheroidal 10	polyhedral 52
Type of abaxial hypoderm cells	polyhedral cuboidal × polyhedral	polyhedral cuboidal: polyhedral spheroidal 3:1	elliptical polyhedral cuboidal 2	polyhedral cuboidal 7	polyhedral spheroidal 68	polyhedral 42
Adaxial type of trichomes	whiplash and glandular present (both)	Both present : glandular absent 4:0	whiplash and glandular 90	glandular absent 29		
Abaxial type of trichomes	whiplash and glandular present (both)	Both present : glandular absent 3:1	whiplash and glandular 110	glandular absent 9		

Table 5.6 Illustrate univariate statistics for leaf anatomical traits for the mapping population. The traits measured included width of lamina (wl), width at vein (wv), width of adaxial and abaxial epidermis (wde & wbe), width of adaxial and abaxial hypodermis (wdh & wbh), width of adaxial cell (wdc), number of adaxial and abaxial hypodermis (Ndhc & Nbhc), width of abaxial and adaxial epidermis (wbe & wde), width of mesophyll (wm), depth of spongy and pallisade mesophyll (dsm & dpm). The depth and widths are measured in mm.

0	Wl	wv	wde	wdh	wdc	Ndhc	wbe	wbh	wbc	Nbhc	wm	dpm	dsm
N	119	119	119	119	119	119	119	119	119	119	119	119	119
Min	0.18	0.29	0.01	0.02	0.01	0	0.01	0.04	0.01	1	0.07	0.02	0.03
Max	0.69	0.83	0.06	0.35	0.21	4	0.05	0.15	0.17	2	0.20	0.09	0.10
Mean	0.44	0.48	0.03	0.17	0.11	1.31	0.03	0.08	0.11	1.03	0.12	0.05	0.06
Std. error	0.01	0.01	0.001	0.006	0.00	0.05	0.001	0.002	0.003	0.01	0.002	0.001	0.001
Variance	0.01	0.01	0.000	0.004	0.00	0.40	0	0.001	0.001	0.03	0.001	0.000	0.000
Stand. dev	0.10	0.11	0.008	0.06	0.03	0.63	0.007	0.02	0.02	0.18	0.02	0.01	0.01
Median	0.45	0.48	0.03	0.16	0.11	1	0.03	0.08	0.11	1	0.12	0.05	0.06
Skewness	-0.08	0.43	0.28	0.40	0.11	1.81	0.32	0.45	-0.06	5.24	0.55	0.81	0.11
Shapiro-Wilk W	0.99	0.97	0.98	0.98	0.99	0.61	0.98	0.97	0.97	0.17	0.97	0.94	0.98
p(normal)	0.81	0.04	0.07	0.18	0.66	2.99E-16	0.21	0.04	0.06	6.56E-23	0.04	0.0001	0.24

5.6 Variation for stomatal traits in F1 hybrids and mapping population:

The trait distributions data for leaf stomatal traits revealed that these traits are determined by the combined effect of more than one pair of genes (Figure 5.9). Several researchers agree that stomatal patterning is unlikely to be controlled by a single gene (Peterson et al., 2010, Bradshaw and Stettler 1995, Wu et al., 1997). This is discussed in chapter 7 in detail.

The univariate statistics (mean, minimum, maximum, standard variation values) for the quantitative traits in the mapping population are presented in detail in Table 5.7. The mapping population displayed variation for number of clusters per mm^2 ranged from 41.7 to 308.3 (Figure 5.9A), sub stomatal cavity width ranged from 0.04 to 0.30 mm (Figure 5.9B), sub stomatal cavity length ranged from 0.04 to 0.26 mm (Figure 5.9C), number of stomata in each cluster ranged from 0.19 to 6 (Figure 5.9D) and average distance between clusters ranged from 0.11 to 0.59 (Figure 5.9E). The range of values observed is well beyond the parental values for most of the measured traits. This suggests a moderate to high transgressive inheritance in both negative and positive senses as the mapping population data display lower and higher values than the parents. The average frequency distribution for the mapping population and parents illustrated in Figure 5.9, show clearly this transgressive inheritance.

The frequency distributions data tested for normality with Shapiro-Wilk test further revealed that none of the traits showed a good fitting to normal distribution and hence were further transformed for QTL analysis.

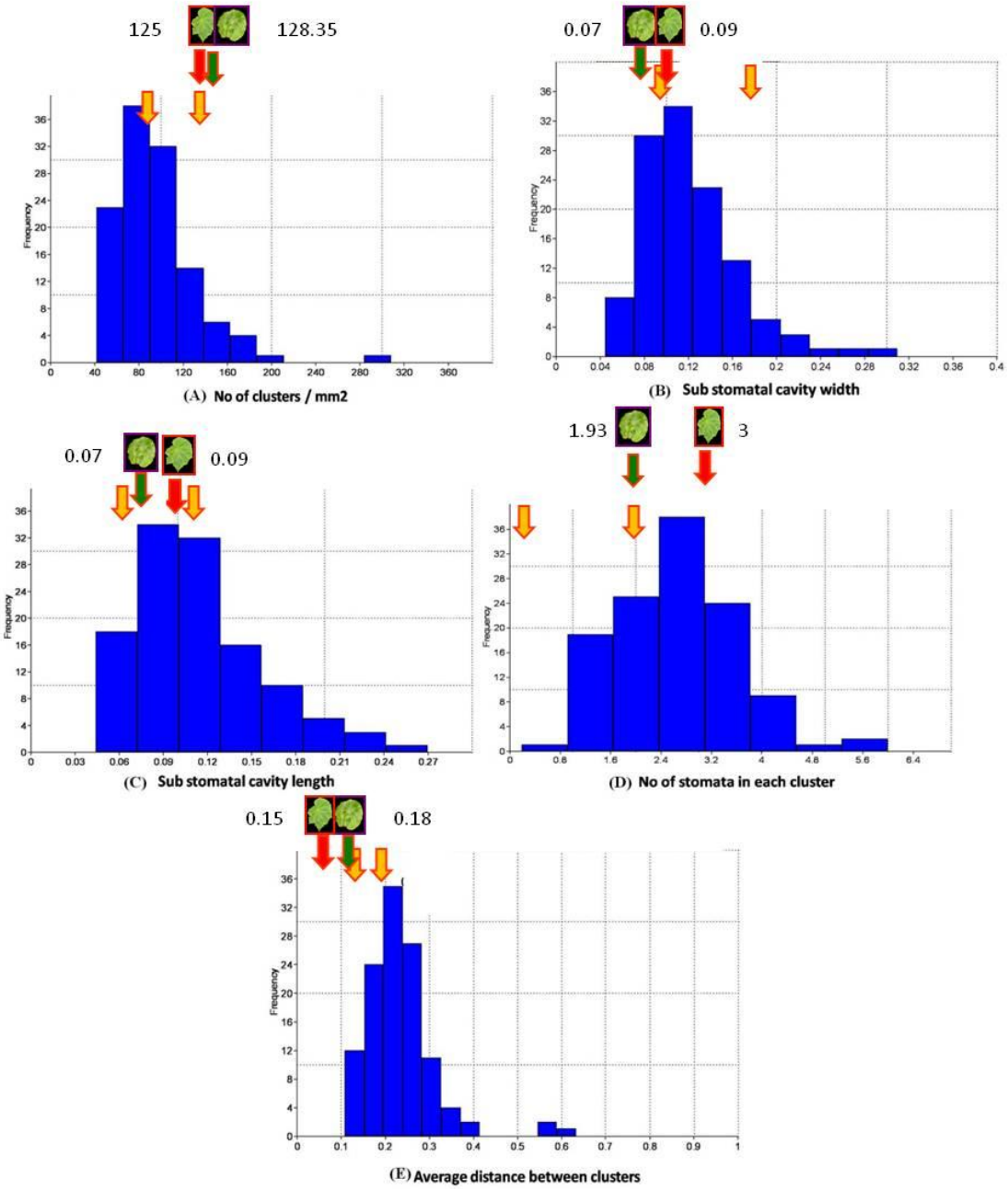

Figure 5.9: Histograms for stomatal clusters per mm^2 (A), sub stomatal cluster width (B), substomatal cluster length (C), number of stomata per cluster (D) and average distance between clusters (E). Arrows are used to point out values for *B. plebeja* marked as red and *B. conchifolia* marked as green as well as range for F1 hybrid family marked as dashed line. A total of 119 individuals were used to generate this statistic

Table 5.7: Univariate statistics for the stomatal traits measured for the mapping population. The traits measured included number of stomatal clusters per mm^2 (NscF), Average distance between clusters (Av), sub stomatal cluster width (sscw), substomatal cluster length (sscl), and number of stomata per cluster (Nsc).

	NscF	Av	sscw	sscl	Nsc
N	119	119	119	119	119
Min	41.70	0.11	0.04	0.04	0.19
Max	308.30	0.59	0.31	0.27	6.00
Sum	11356.6	27.92	14.54	13.62	313.6
Mean	95.43	0.23	0.12	0.11	2.64
Std. error	3.45	0.007	0.00	0.00	0.09
Variance	1420.2	0.006	0.00	0.00	0.98
Stand. dev	37.69	0.07	0.04	0.04	0.99
Median	90.00	0.22	0.11	0.11	2.60
25 prcntil	68.30	0.19	0.09	0.08	1.80
75 prcntil	113.3	0.27	0.15	0.14	3.20
Skewness	1.91	1.99	1.33	0.87	0.54
Kurtosis	7.78	6.54	2.96	0.81	0.80
Geom. mean	89.26	0.22	0.12	0.11	2.43
Shapiro-Wilk W	0.87	0.84	0.91	0.95	0.97
P (normal)	1.80E-08	8.89E-10	2.29E-06	0.0003	0.01

5.7 Variation for leaf trichome traits in F1 hybrids and mapping population:

The segregation data for qualitative traits revealed that traits such as type of trichomes are not under simple genetic control (Table 5.5). The trait distributions data for quantitative traits also revealed that leaf trichome traits are determined by the combined effect of more than one pair of genes (Table 5.8; Figure 5.10, 5.11). There is enough evidence that suggests that trichome patterning traits are controlled by a number of genes (Grebe, 2012; Galway, 1994; Koornneef, 1981; Hulskamp et al, 1994). This is discussed in chapter 7 in detail.

The univariate statistics (mean, minimum, maximum, standard variation values) for the quantitative traits in the mapping population are presented in detail in Table 5.8. The number of abaxial whiplash trichomes ranged from 1 to 42.6 (Figure 5.10B), length of abaxial whiplash trichomes ranged from 0.44 to 3.33 mm (Figure 5.10C), number of abaxial glandular trichomes ranged from 5 to 213.3 (Figure 5.10D), length of Abaxial glandular trichomes ranged from 0.01 - 0.27 (Figure 5.10E), number of adaxial whiplash trichomes ranged from 0.67 to 16.67 (Figure 5.11B), length of adaxial whiplash trichomes ranged from 0.47 to 2.33 (Figure 5.11C), length of adaxial glandular trichomes in the range of 0.01 to 0.32 mm (Figure 5.11A), number of adaxial glandular trichomes ranged from 0 to 4 (Figure 5.11D) within the mapping population. The range of values observed is well beyond the parental values for most of the measured traits. This suggests a moderate to high transgressive inheritance in both negative and positive senses as the mapping population data display lower and higher values than the parents. The average frequency distribution for the mapping population and parents illustrated in Figure 5.2, show clearly this transgressive inheritance.

The frequency distributions data tested for normality with Shapiro-Wilk test further revealed that none of the traits showed a good fitting to normal distribution. The traits that were not normally distributed were further transformed for QTL analysis.

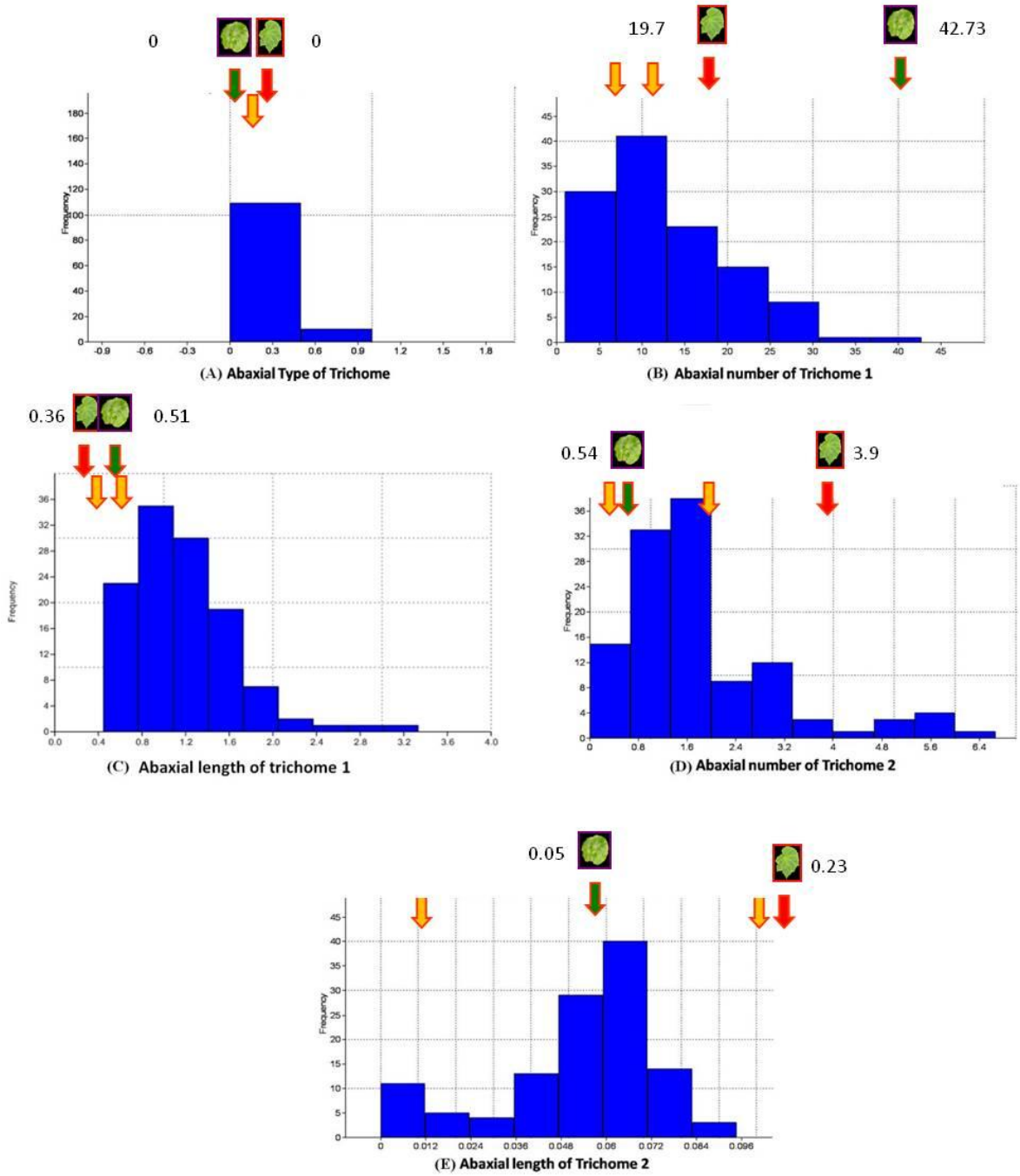

Figure 5.10: Histograms for abaxial trichome traits. The traits measured included abaxial type of trichome (A), abaxial number of trichome 1 (B), abaxial length of trichome 1 (C), abaxial number of trichome 2 (bNt2) and abaxial length of trichome 2 (E). Arrows are used to point out values for *B. plebeja* marked as red and *B. conchifolia* marked as green as well as range for F1 hybrid family marked as dashed line. A total of 119 individuals were used to generate this statistic.

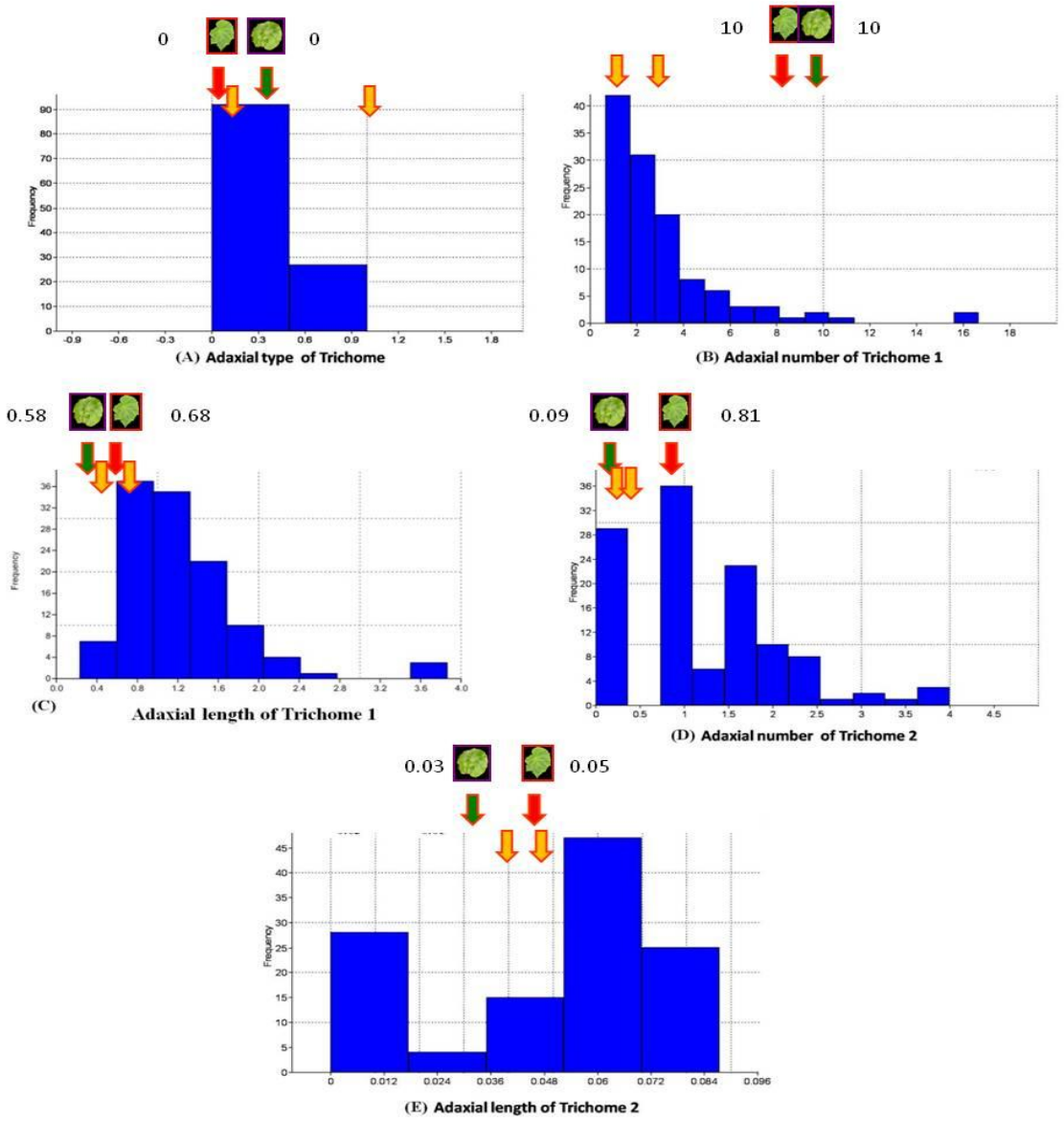

Figure 5.11: Histograms for adaxial trichome traits. The traits measured included adaxial type of trichome (A), adaxial number of trichome 1 (B), adaxial length of trichome 1 (C), adaxial number of trichome 2 (D) and adaxial length of trichome 2 (E). Arrows are used to point out values for *B. plebeja* marked as red and *B. conchifolia* marked as green as well as range for F1 hybrid family marked as dashed line. A total of 119 individuals were used to generate this statistic.

Table 5.8: Univariate statistics for the trichome related traits measured for the mapping population. The traits measured included univariate statistics for the trichome related traits measured for the mapping population. The traits measured included abaxial length of trichome 1 (blt1), abaxial number of trichome 1 (bNt1), abaxial length of trichome 2 (blt2), abaxial number of trichome 2 (bNt2), adaxial length of trichome 1 (dlt1), adaxial number of trichome 1 (dNt1), adaxial length of trichome 2 (dlt2), adaxial number of trichome 2 (dNt2). The lengths and widths are measured in mm.

	blt1	bNt1	blt2	bNt2	dlt1	dNt1	dlt2	dNt2
N	119	119	107	119	119	119	91	119
Min	0.447	1	0.012	0	0.23	0.33	0.01	0
Max	3.33	42.67	0.273	6.67	3.86	16.67	0.30	3.67
Mean	1.17	12.46	0.054	1.57	1.22	3.08	0.05	0.86
Std. error	0.04	0.73	0.002	0.12	0.06	0.25	0.004	0.07
Variance	0.23	62.56	0.0009	1.86	0.36	7.30	0.002	0.65
Stand. dev	0.48	7.91	0.03	1.36	0.60	2.70	0.04	0.81
Median	1.125	10.67	0.05	1.33	1.08	2.33	0.05	0.67
Skewness	1.38	0.98	4.01	1.49	1.94	2.81	3.35	1.03
Shapiro-Wilk W	0.91	0.93	0.6741	0.86	0.84	0.71	0.633	0.88
p(normal)	1.17E-06	2.65E-05	4.43E-14	6.07E-09	1.17E-09	5.21E-14	9.57E-14	5.85E-08

5.8 Variation for traits related to vascular patterning in F1 hybrids and mapping population:

The trait distributions data for leaf anatomical traits revealed that these traits are determined by the combined effect of more than one pair of genes (Figure 5.12). Researchers agree that vascular patterning traits are controlled by a number of genes (Carland and McHale, 1996; Cnops et al., 1996; Berleth and Jurgens, 1993; Przemeck et al., 1996). This is discussed in chapter 7 in detail.

The mapping population displayed variation for number of central vascular bundles, number of inner vascular bundles, number of outer vascular bundles, width of inner vascular bundles, depth of inner vascular bundles, width of outer vascular bundles, depth of outer vascular bundles (Table 5.9; Figure 5.12). The number of central vascular bundles ranged from 0.15 and 8.5 (Figure 5.12A), number of inner vascular bundles ranged from 4 to 8 (Figure 5.12 B), number of outer vascular bundles ranged from eight to thirty three (Figure 5.12C), width of inner vascular bundles ranged from 0.05 to 0.34 mm (Figure 5.12D), depth of inner vascular bundles ranged from 0.16 to 0.74 mm(Figure 5.12E), width of outer vascular bundles ranged from 0.05 to 0.27 mm (Figure 5.12F) and depth of outer vascular bundles ranged from 0.16 to 0.21 mm (Figure 5.13G) within the mapping population. The range of values observed is well beyond the parental values for most of the measured traits. This suggests a moderate to high transgressive inheritance in both negative and positive senses as the mapping population data display lower and higher values than the parents. The average frequency distribution for the mapping population and parents illustrated in Figure 5.2, show clearly this transgressive inheritance.

The frequency distributions data tested for normality with Shapiro-Wilk test further revealed that three out of eight traits showed a good fitting to normal distribution. The five traits that were not normally distributed were further transformed for QTL analysis.

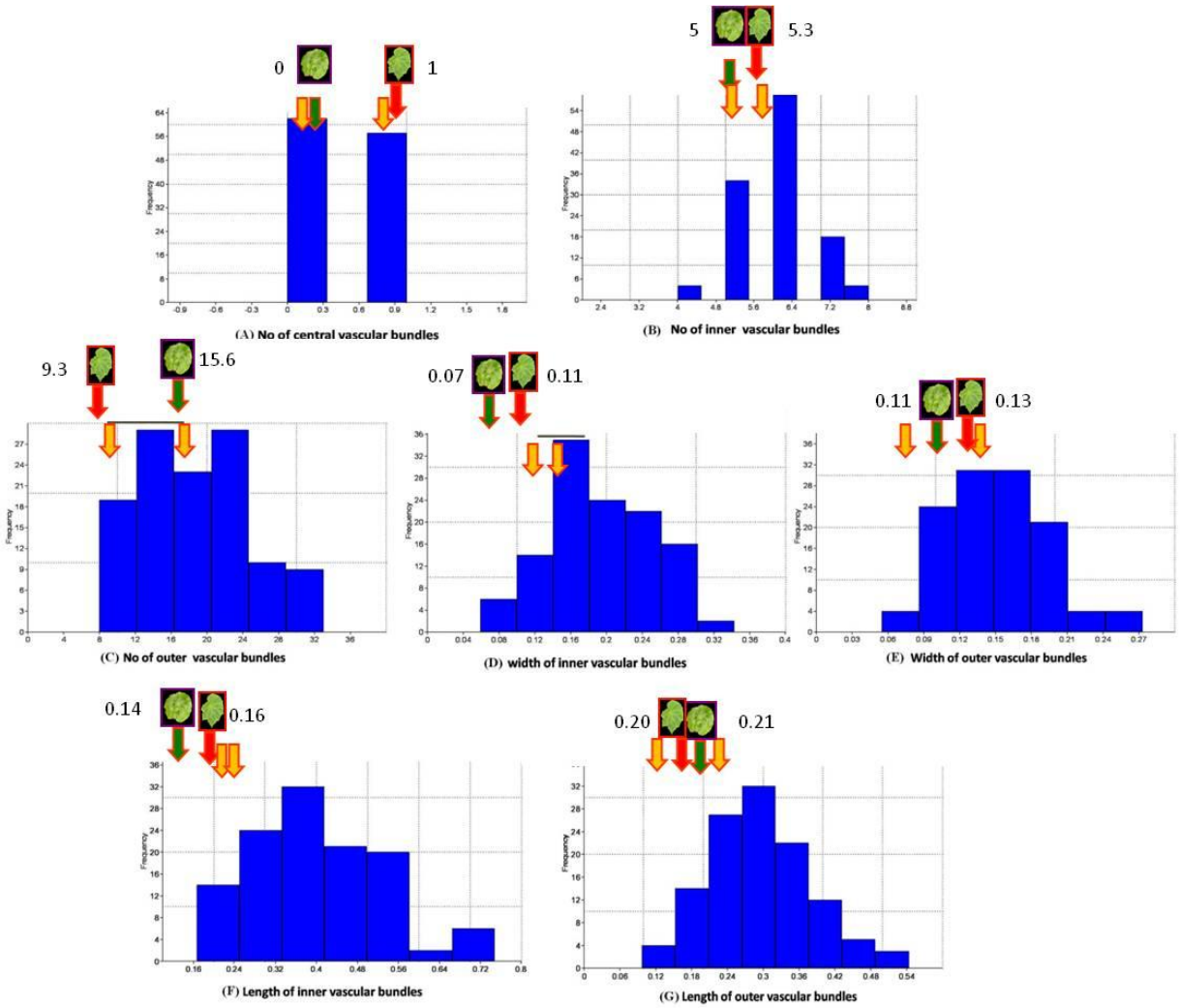

Figure 5.12: Illustrate histograms for number of central vascular bundle (A), number of inner vascular bundles (B), number of outer vascular bundles (C), width of inner vascular bundles (D), width of outer vascular bundles (E), length of inner vascular bundles (F), Length of outer vascular bundles (G). Arrows are used to point out values for *B. plebeja* marked as red and *B. conchifolia* marked as green as well as range for F1 hybrid family marked as dashed line. A total of 119 individuals were used to generate this statistic.

Table 5.9: Univariate statistics for traits related to vascularisation patterning in the mapping population. The traits measured included width of petiole (wp), number of central vascular bundle (Ncvb), number of inner vascular bundles (Nivb), number of outer vascular bundles (Novb), length of inner vascular bundles (Livb), width of inner vascular bundles (wivb), Length of outer vascular bundles (Lovb), width of outer vascular bundles (wovb). The length and width of inner and outer vascular bundles is measured in mm.

0	wp	Ncvb	Nivb	Novb	Livb	wivb	Lovb	wovb
N	119	119	119	119	119	119	119	119
Min	1.79	0	4	8	0.16	0.05	0.09	0.05
Max	7.95	1	8	33	0.74	0.34	0.54	0.27
Mean	4.93	0.47	5.86	18.82	0.40	0.19	0.29	0.15
Std. error	0.13	0.04	0.07	0.54	0.01	0.005	0.007	0.003
Variance	2.09	0.25	0.69	35.75	0.01	0.003	0.007	0.002
Stand. dev	1.44	0.50	0.83	5.97	0.12	0.05	0.08	0.04
Median	5.06	0	6	18	0.39	0.18	0.29	0.15
Skewness	-0.29	0.08	0.25	0.32	0.52	0.14	0.28	0.41
Shapiro-Wilk W	0.97	0.63	0.87	0.97	0.97	0.98	0.98	0.98
p(normal)	0.03	9.38E-16	9.39E-09	0.022	0.01	0.44	0.45	0.10

5.9 Variation for functional traits in F1 hybrids and mapping population:

The trait distributions data for leaf functional traits revealed that these traits are determined by the combined effect of more than one pair of genes (Figure 5.13). There is ample evidence that suggests that functional traits are unlikely to be controlled by a single gene (Teng et al., 2004; Zhao et al., 2008; Adachi et al., 2011; Gentzbittel et al., 2001). This is discussed in chapter 7 in detail.

The functional trait univariate statistics (mean, minimum, maximum) for the mapping population are shown in Table 5.10. The net assimilation rate ranged from 0.15 µmol m^{-2} s^{-1} and 8.5 µmol m^{-2} s^{-1} (Figure 5.13A), stomatal conductance ranged from 7.62 mmol m^{-2} s^{-1} and 288 mmol m^{-2} s^{-1} (Figure 5.13B), total nitrogen content ranged from 1.35 percent to 6.31 percent (Figure 5.13C), total carbon content ranged from 26.8 percent and 72.6 percent, total leaf dry matter content ranged from 4.36 mg g^{-1} and 121.6 mg g^{-1} (Figure 5.13E), leaf mass area ranged from 3.61 g m^{-2} and 42.09 g m^{-2} (Figure 5.13F) and chlorophyll content ranged from 21.47 spad unit and 59.8 spad units (Figure 5.13G) within the mapping population. This suggests a moderate to high transgressive inheritance in both negative and positive senses as the mapping population data display lower and higher values than the parents (Figure 5.13). Interestingly, transgression beyond the parental values within the mapping population was also observed for traits including those for which parental values hardly differed, such as net assimilation rate and leaf dry matter content. The average frequency distribution for the mapping population and parents illustrated in Figure 5.13, show clearly this transgressive inheritance.

The frequency distributions data tested for normality with Shapiro-Wilk test further revealed that three out of seven showed a good fitting to normal distribution. The traits that were not normally distributed were further transformed for QTL analysis.

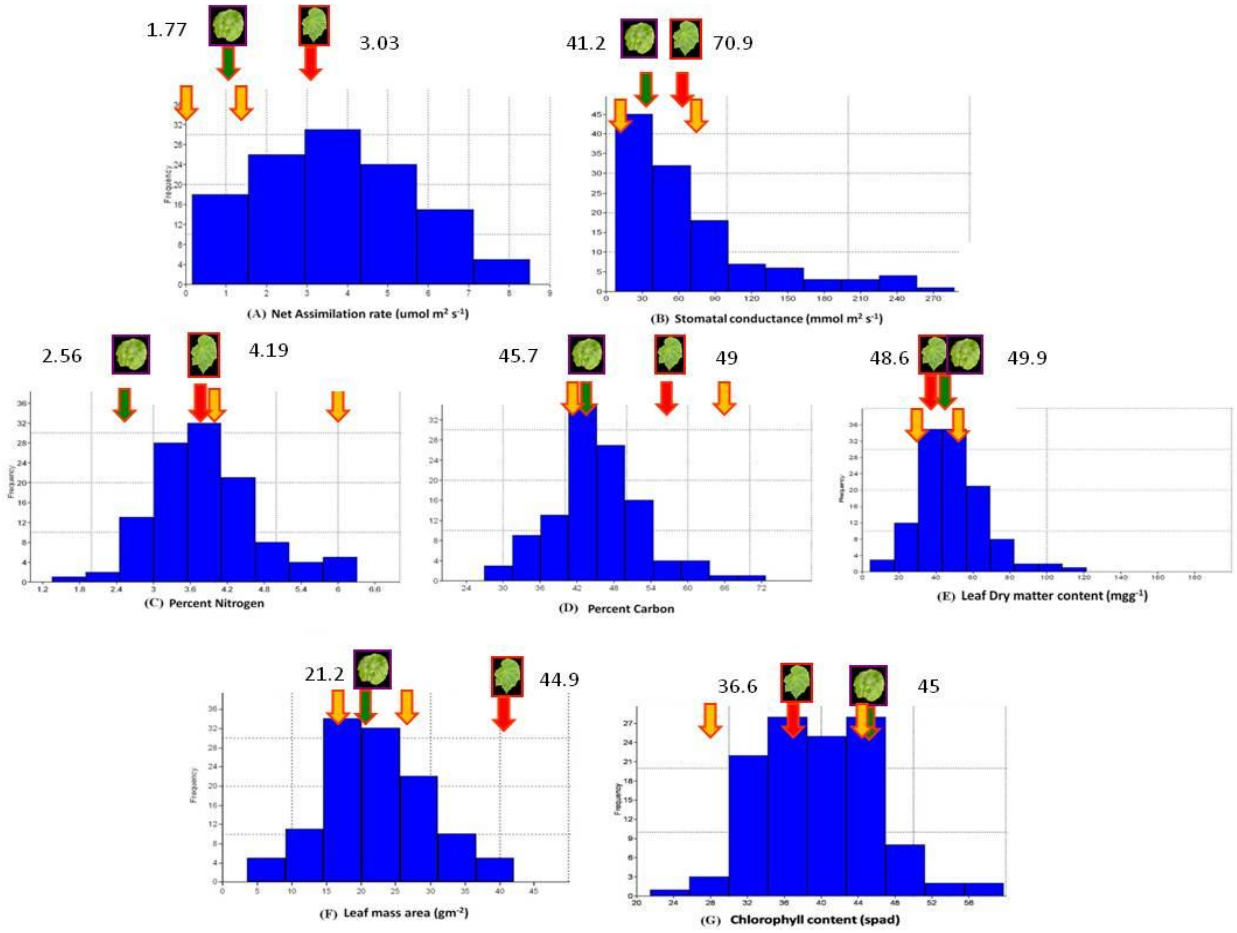

Figure 5.13: Illustrate histograms for net assimilation rate (A), stomatal conductance (B), nitrogen (C) and carbon content (D), specific leaf area (SLA), leaf dry matter content (Ldmc), Leaf mass area (LMA), chlorophyll content (Spad). Arrows are used to point out values for *B. plebeja* marked as red and *B. conchifolia* marked as green as well as range for F1 hybrid family marked as dashed line. A total of 119 individuals were used to generate this statistic.

Table 5.10: Univariate statistics for the functional and resource use strategy traits measured for the mapping population. The traits measured included Net assimilation rate (A_{max}), stomatal conductance (mmol m^{-2} s^{-1}), leaf dry matter content (mg g^{-1}), Leaf mass Area (g m^{-2}), chlorophyll content (Spad), nitrogen (%) and carbon content (%).

	A_{max}	Cond	Ldmc	LMA	Spad	N	C
N	119	119	119	119	119	119	119
Min	0.15	7.62	4.36	3.61	21.47	1.34	26.87
Max	8.51	288.0	121.6	42.09	59.80	6.30	72.67
Mean	3.71	67.66	48.90	22.30	39.48	3.86	45.17
Variance	3.69	3527.1	345.82	57.58	39.38	0.76	55.55
Stand. dev	1.92	59.38	18.59	7.58	6.27	0.87	7.45
Median	3.57	45.90	45.18	21.94	39.2	3.73	44.90
Skewness	0.26	1.67	0.85	0.26	0.25	0.40	0.38
Shapiro -Wilk W	0.98	0.80	0.95	0.98	0.98	0.98	0.98
p(normal)	0.08	2.48E-11	4.86E-04	0.23	0.53	0.08	0.13

5.10 Conclusions:

The study of the inheritance patterns of the traits revealed that a few traits such as colour of stipules, margin colour strength and absence/presence of petiole base might be controlled by single genes. There is ample evidence in the literature confirming the monogenic nature of anthocyanin pigmentation traits in Asiatic hybrid lily, Caladium, and Medicago (Gutschick, 1999; Yamagishi et al., 2003; Singh et al., 1976). The anthocyanin pigmentation traits also seem to be under strong environmental effects of shading and hence made it difficult to analyse the inheritance pattern of these traits in the backcross population.

The trait distributions data revealed that most of the measured traits are determined by the combined effect of more than one pair of genes. Traits such as leaf mass area, chlorophyll content, length and width of leaf, and length of outer vascular bundles were normally distributed i.e., the data was evenly distributed around the mapping population mean. However the trait distributions revealed that the distributions for most of the continuous traits measured in the mapping population showed a moderate to high transgressive inheritance in both negative and positive senses as the trait data for the mapping population displayed lower and higher trait values than the parents. Surprisingly, the trait transgression seems to be fairly evenly divided among trait categories such as morphology, ecophysiology and anatomy.

Based on the results, I can conclude that niche divergence in *Begonia* could be achieved by the evolution of extreme morphological and ecophysiological features. However, to verify glass house results this needs to be tested by measuring the hybrid traits in the natural habitat of Mexico. The niche divergence could further lead to the development of reproductive isolation between a hybrid lineage and its progenitors.

The occurrence of natural hybrids (Peng & Ku, 2009; Peng & Sue, 2000; Peng et al., 2010), the presence of weak reproductive barriers among them (Twyford et al., 2012) and the presence of transgressive segregation in an F1 hybrid family and in an F1BC1 hybrid population suggested the potential for hybrid speciation in genus *Begonia.*

Chapter 7 presents quantitative trait loci (QTLs) mapping results for the ecophysiological and morphological traits that were measured for the mapping population and gives us some insights about the genetic bases of transgressive traits in the F1BC1 hybrid family between *B. plebeja* and *B. conchifolia.*

CHAPTER 6. Correlation analysis: Linkage or adaptation?

6.1 Adaptation or correlated evolution of traits in *Begonia* section Gireoudia:

The traits I have been analysing (such as leaf shape, photosynthetic function and patterns of surface features such as trichomes and stomata) I have dealt with separately, while in reality, leaves function as integrated structures. Combinations of traits occur non-randomly, because of selection for their coordinated function, or they may be linked through common developmental pathways or there might be a common genetic basis for their correlated evolution (Midgely and Bond, 1989). Many suites of characters may be functionally integrated with each other and are probably not acquired independently. Other traits including the size of organs such as the size of leaf and over all plant size may have a common genetic and developmental basis in addition to functional integration (Midgely and Bond, 1989).

Evidence in the literature also supports the coordinated evolution of leaf form and function (Givnish and Vermeij, 1976; Fonseca et al., 2000; Wright et al., 2004). For example, leaf size is generally small in habitats that are characterised by low moisture and nutrient availability as this reduces boundary layer resistance preventing overheating and allowing higher photosynthetic water-use efficiency. Other adaptations to dry, infertile environments are dense trichomes on leaves, high SLA, leaf dissection, long leaf lifespan, and these traits would appear correlated with leaf size along a humidity/fertility gradient (Parkhurst and Loucks, 1972; Cunningham et al., 1999; Fonseca et al., 2000).

The correlated nature of traits has not only been observed at the habitat level but has also been identified at the global level. These relationships observed at the global level constitute the universal leaf economics spectrum and illustrates the range of adaptive strategies adopted by leaves (Wright et al., 2004). The spectrum links high photosynthetic ability (A_{mass}) with high nitrogen content (N_{mass}), short leaf life span (LL), and low leaf mass area (LMA). Leaves with high photosynthetic ability require large inputs of nitrogen to create the pools of enzymes and pigments needed to sustain high rates of CO_2 uptake (Field and Mooney, 1986). Leaves with high photosynthetic ability are also marked by short leaf life spans and low leaf mass areas. These correlations reflect underlying adaptive constraints on plant trait evolution (Wright et al., 2004). For example, selection does not seem to result in high net assimilation rates in long lived leaves, although this would obviously be beneficial to the plant. To become long lived leaves must be physically tough and have strong defences against herbivores. Investments in these precautions (such as thick leaves, secondary compounds, waxy cuticles, layers of trichomes) can inhibit optimum photosynthetic activity (Villar et al., 2001; Delucia et al., 2010).

Correlations between various attributes also suggest a common genetic basis for the correlated evolution of those attributes (Davis, 2001). Two possible mechanisms for these correlations are pleiotropy and genetic linkages. Pleiotropy is the association of more than one phenotype with a single genotype, and linkage involves the inheritance of independent genes that determine different traits together as a result of their close proximity to each other in the genome (Falconer and Mackay, 1996).

6.2 Objectives

This chapter presents patterns of multiple trait correlations by studying leaf morphological, ecophysiological and micromorphological traits across species of *Begonia* in section

Gireoudia and in the offspring of a backcross between *B. plebeja* and *B. conchifolia*. For multiple trait correlations there is not a widely accepted method. Therefore the analysis was carried out with a number of methods to analyse patterns of correlations in the species and mapping population matrix (a) The strength and direction of correlations in the mapping population were analysed using spearman rank order correlations in R software. A critical P-level of 0.05 was used as a cut-off for significance. Most of the correlations observed were also confirmed at P value of 0.001. Heat maps were generated in R software to look for similarity between the species and mapping population matrix using the following code (Appendix 6.2) (b) The variance of the eigenvectors was also investigated to confirm presence of correlations in a principal components analysis using PAST software. (c) Three way trait relationships were carried out to investigate trends displayed by the global leaf economics spectrum. The Hierarchical cluster analysis based on Ward's (1963) method were carried out in PAST software.

This chapter investigates:

1. Do patterns of trait correlations in the *Begonia* section Gireoudia mirror those seen in the world wide leaf economics spectrum (Wright et al., 2004)?
2. Are there adaptive suites of trait identifiable in section Gireoudia?
3. Are traits correlated in across section Gireoudia also correlated in the mapping population, suggesting genetic (developmental or pleitropic linkage) rather than the action of selection?

6.3 Results:

6.3.1 Species correlation matrices:

There were many significant pairwise correlations between traits in the species correlation matrix suggesting that the traits are either correlated because they are developmentally constrained (e.g., linked or pleiotropic) or because they have been selected together. The

different categories of traits selected together might ensure better performance of species in their respective habitats (Supplementary Table 1).

The correlations revealed interesting patterns that exist at the sectional level, for example, the variation among *Begonia* species in stomatal conductance and positive correlations of this trait with net assimilation rate reflects the gas exchange limitation of photosynthesis (r^2 = 0.48, $P < 0{\cdot}05$). Net assimilation rate and stomatal conductance are closely correlated as assimilation is dependent on open stomata (Salisbury and Ross, 1992; Meng and Arp, 1992). However, certain ecologically important relationships established in other studies such as that of the linkage of high A_{max} with high N_{mass} do not exist across *Begonia* species. The relationship of these two traits has been confirmed for most of the taxa (Wright, 2004). Similarly, no significant relationship was detected between net assimilation rate and chlorophyll content (as measured by SPAD) across the species, neither was a high A_{max}-low LMA relationship found across species in section Gireoudia, although is commonly reported (Poorter et al., 2009; Grime, 2001; Westoby et al., 2002),

An interesting pattern observed in the species correlation matrix was the correlations between leaf size and shape traits with vascular traits. Leaf size was significantly correlated with the cross-sectional area of petiole (*r2* = 0.80, $P < 0{\cdot}05$), indicating that thick stems are holding more leaf area, and thicker petioles (Corner, 1949; Westoby and Wright, 2003; Sun *et al.*, 2006). *Begonia* species that have compound leaves usually support a larger lamina area for a given petiole size and are usually larger in both lamina size and petiole size than simple leaf species.

I found a significant correlation between leaf area and width of stomatal cluster (*r2* = 0.55, $P < 0{\cdot}05$). Leaf size is considered as an indicator of biomass production and its positive

correlation with substomatal cluster width might affect biomass accumulation in *Begonias*. In other words large stomatal clusters are more efficient at supplying the leaf with CO_2, or promoting efficient transpiration than evenly single stomata.

Significant positive correlations were also found between leaf shape and the width of vascular bundles. Leaf shape traits were correlated with abaxial and adaxial trichome density and abaxial and adaxial length of trichomes suggesting that the correlated evolution of leaf shape and trichome traits is developmentally constrained in *Begonia* section Gireoudia. A few papers report mutants with effects on both leaf shape and trichome presence or type (Cho et al., 2005). Hormones are also known to have an effect on both leaf shape and trichomes. In a study conducted on *B. dregei* it was speculated that increased gibberellin concentration had caused an increase in the number of sinus trichomes and more deeply lobed leaves (Mclellan, 2005).

Leaf anatomy was also found to have significant positive correlations with several functional traits. Chlorophyll content was found to be significantly correlated with depth of adaxial hypodermis (r^2 = 0.45, P < 0.05) and number of adaxial hypodermal cells (r^2 = 0.50, P < 0.05). It seems that *Begonia* species make a higher investment in chlorophyll content to capture light in the thick leaves. This effort is reinforced by the multilayered hypodermis and its cells that have been suggested to focus light onto the mid-leaf band of photosynthetic cells in *Begonia* species (r^2 = 0.45, P < 0.05).

A positive relationship was also noticed for average distance between cluster size and multiple hypodermis (r^2 = 0.49, P < 0.05) suggesting that these two traits might be related to drought adaptation in *Begonia* species. Previous studies have also confirmed this relationship in *Begonia* species. Hoover (1986) studied the relationship between stomatal clustering traits

and habitat attributes of *B. nelumbifolia* and *B. heracleifolia.* An interesting finding of his study was that populations growing on rocks near waterfalls had larger stomatal clusters. This suggested the possible involvement of these clusters in water conservation. Tang and his colleagues also reported a positive relationship between cluster size and water filled multiple hypodermis in drought adapted *Begonia peltatifolia* (Tang et al., 2002). Bardley and colleagues also observed a correlation between the presence of clustered stomata and multiple hypodermis in a study conducted on 16 *Begonia* species suggesting the obvious role of multiple hypodermis in preventing excessive water dissipation (Fahn, 1990).

The correlation analysis also provided strong evidence that the development of veins and stomata in *Begonia* are coordinated. Leaf stomatal density was significantly correlated with the number of inner vascular bundles ($r^2 = 0.46$, $P < 0.05$) and dimensions of inner and outer vascular bundles (supplementary data). Stomata are responsible for both leaf CO_2 uptake and transpiration. The strong coordination of vascular bundle traits and stomatal density suggests that some linking developmental process is guiding the optimal development of vascular bundles to match the stomatal demand for water.

6.3.2 Mapping population correlation matrix

To identify if the correlation of traits in the species matrix had a genetic basis, the mapping population was used to assess the significant correlations in the species correlation matrix that were also found in the mapping population matrix suggesting that the traits are genetically linked. Heat map for the species (a) and the backcross population (b) showing spearman rank order correlation coefficients for functional, leaf size and shape, leaf anatomy, and morphological traits are shown in Figure 6.2.

Figure 6.1 presents a schematic diagram of the trait-trait relationships present in both the matrixes. For example, the relationship between A_{max} and conductance was significant and positive across all species (r^2 = 0·48, P < 0·05, n = 21), and was strongly significant within the mapping population (r^2 = 0.90, P < 0.05, n = 115) suggesting that both the traits are not only functionally linked but might have a genetic basis to the correlation). Similarly, leaf size was significantly correlated with the cross-sectional area of petiole across species (*r2* = 0.80, *P* < 0·05) and in the mapping population (r^2 = 0.80, *P* < 0·57) suggesting the strong linkage of leaf size and cross-sectional area of petiole.

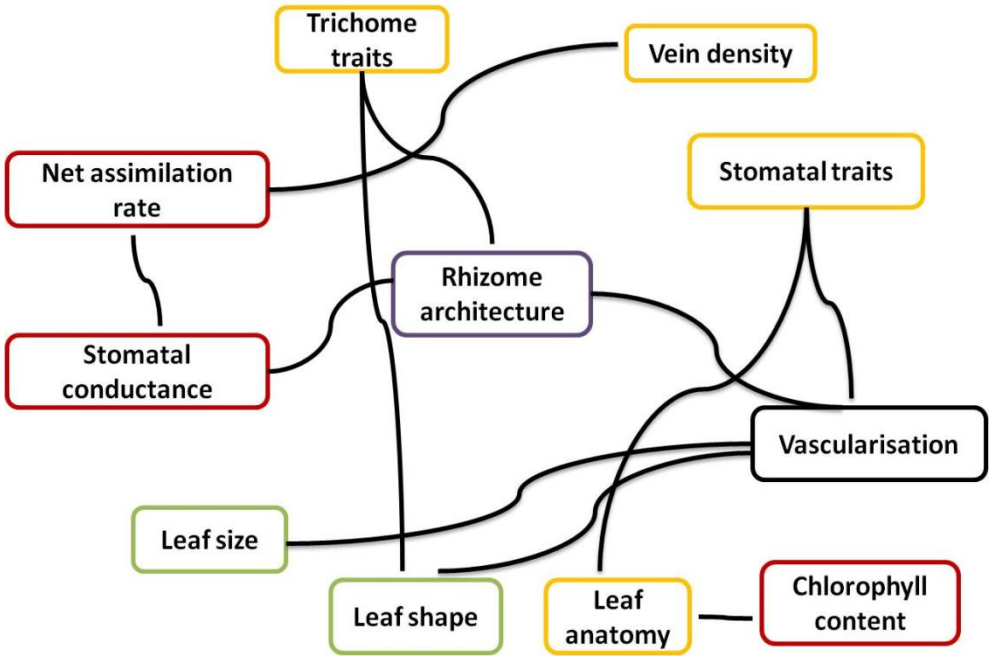

Figure 6.1: Schematic diagram of the trait-trait relationships significant in both the species and mapping population matrices.

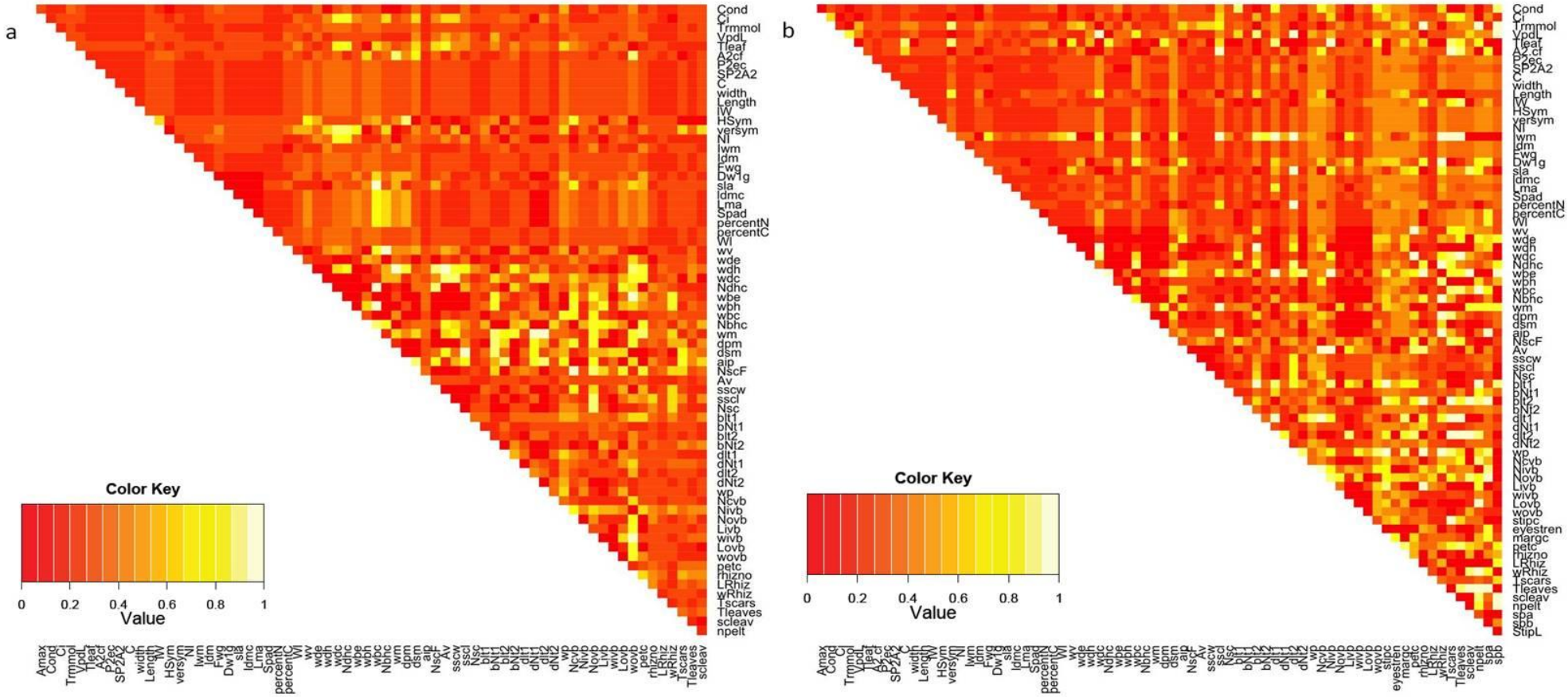

Figure 6.2: Heat map for the species (a) and the backcross population (b) showing spearman rank order correlation coefficients for functional, leaf size and shape, leaf anatomy, and morphological traits. Numerical values are shown in Supplementary Table.

6.3. *Begonia* with reference to the global leaf economics spectrum:

The documentation of the interspecific variation in *Begonia* section Gireoudia suggested that *Begonia*s are distinctive in their low LMA and low gas exchange values (chapter 3). It is now clear that *Begonias* have reduced requirement for investments in dry mass per unit photosynthetic leaf area. However what is unclear is why *Begonias* lack correlations between high A_{max} and high $N_{mass,}$ and high A_{max} and low LMA seen in most other species (Figure 6.3). One explanation for the absence of these globally observed patterns across *Begonia* section Gireoudia could be due to the small data set used here as well as the small range of variation observed for these traits. The world wide leaf economics spectrum was generated with 2,548 species from across the world and the traits varied by one to two orders of magnitude across the data set. In order to detect the broad relationships among *Begonia* species in section Gireoudia the range of variation should have been large or at least one order of magnitude (Wright et al., 2004).

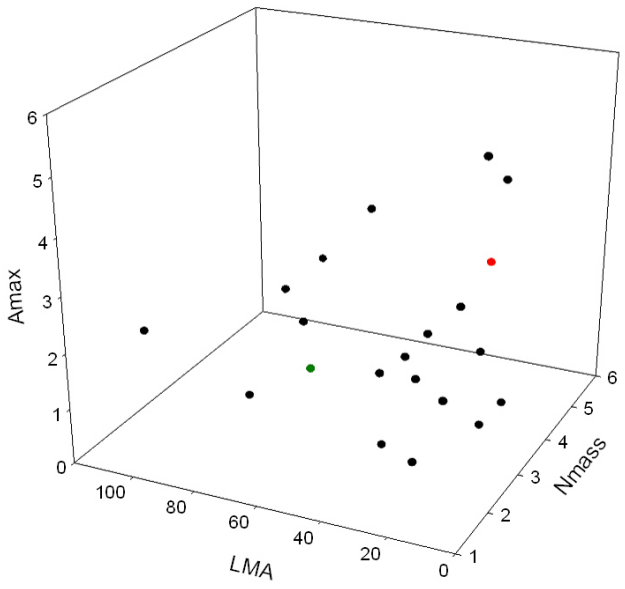

Figure 6.3: Three-way trait relationship graph for species of *Begonia* section Gireoudia generated with net assimilation rate (µmol m^{-2} s^{-1}), leaf mass area (g m^{-2}) and Nitrogen (%). *B. plebeja* is represented by the red dot while *B. conchifolia* is presented by the green dot.

6.4 Hierarchical cluster analysis:

The suites of traits measured for the species in *Begonia* section Gireoudia were also subjected to hierarchical cluster analysis based on Wards method in PAST software (Hammer et al., 2001) (Figure 6.4). Ward's method was an appropriate method to apply since it uses an analysis of variance approach to evaluate the distances between clusters (Ward, 1963). To control for the range of trait values data was standardized before analysis. The resulting dendrogram resolved two distinct groups for the species in section Gireoudia based on leaf size and shape.

Comparison of Molecular and Phenotypic cluster analysis:

Cluster analysis classified *Begonia* species into two main groups. Comparison of the molecular phylogeny for Central American *Begonia* species (Nichola Harrison unpublished) and the phenotypic cluster tree revealed more compelling evidence that closely related species have undergone extensive phenotypic divergence in order to adapt to their respective habitats (Figure 6.6). Figure 6.5 present clustering of traits that are correlated with ..eachother and did not provide much information. To investigate patterns of correlations further I did a principal component analysis.

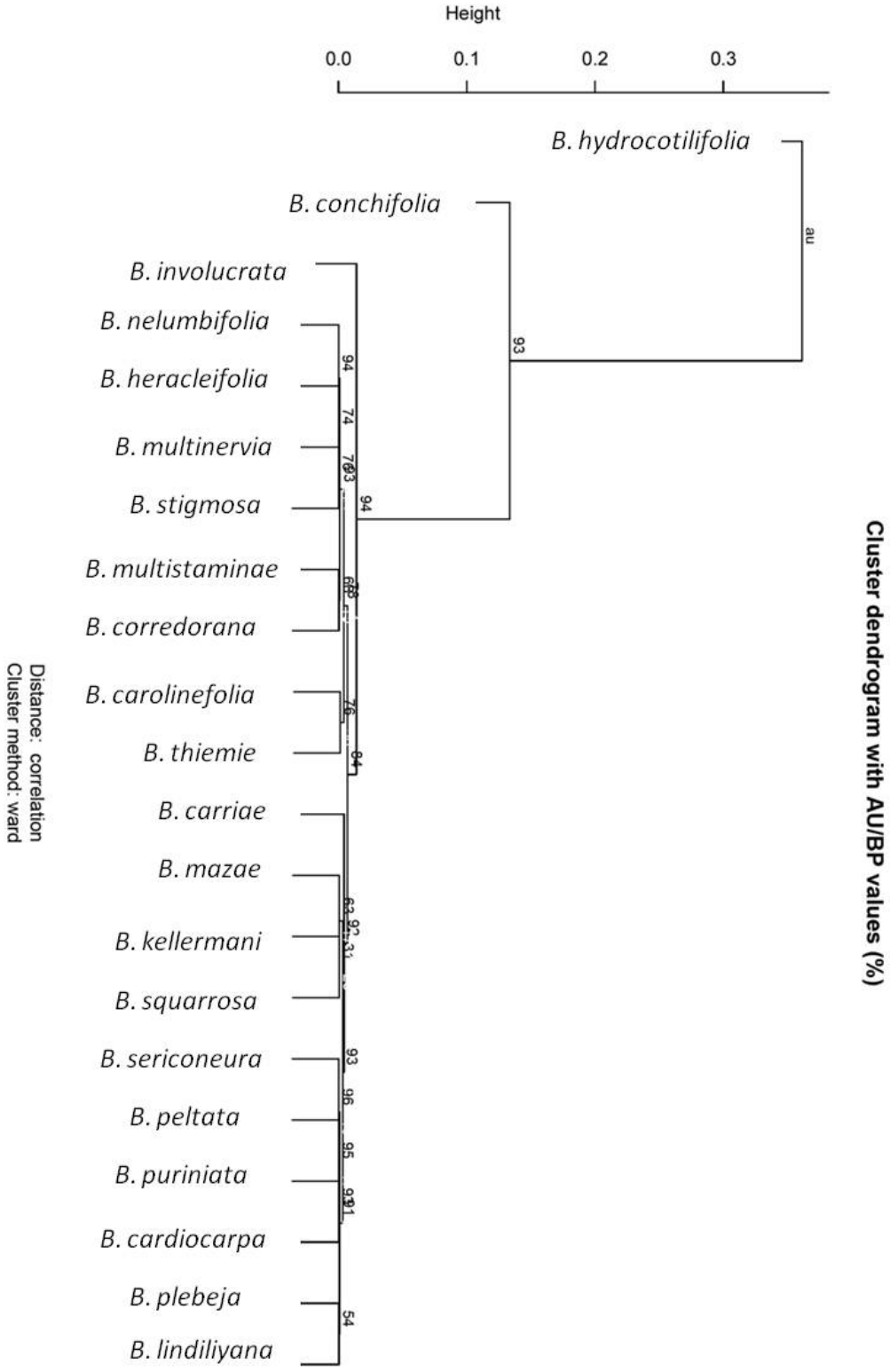

Figure 6.4: Dendrogram of the twenty one species of *Begonia* based on leaf morphological traits, made using correlation distances. The number on the nodes represents the support values based on 10,000 bootstrap replicas.

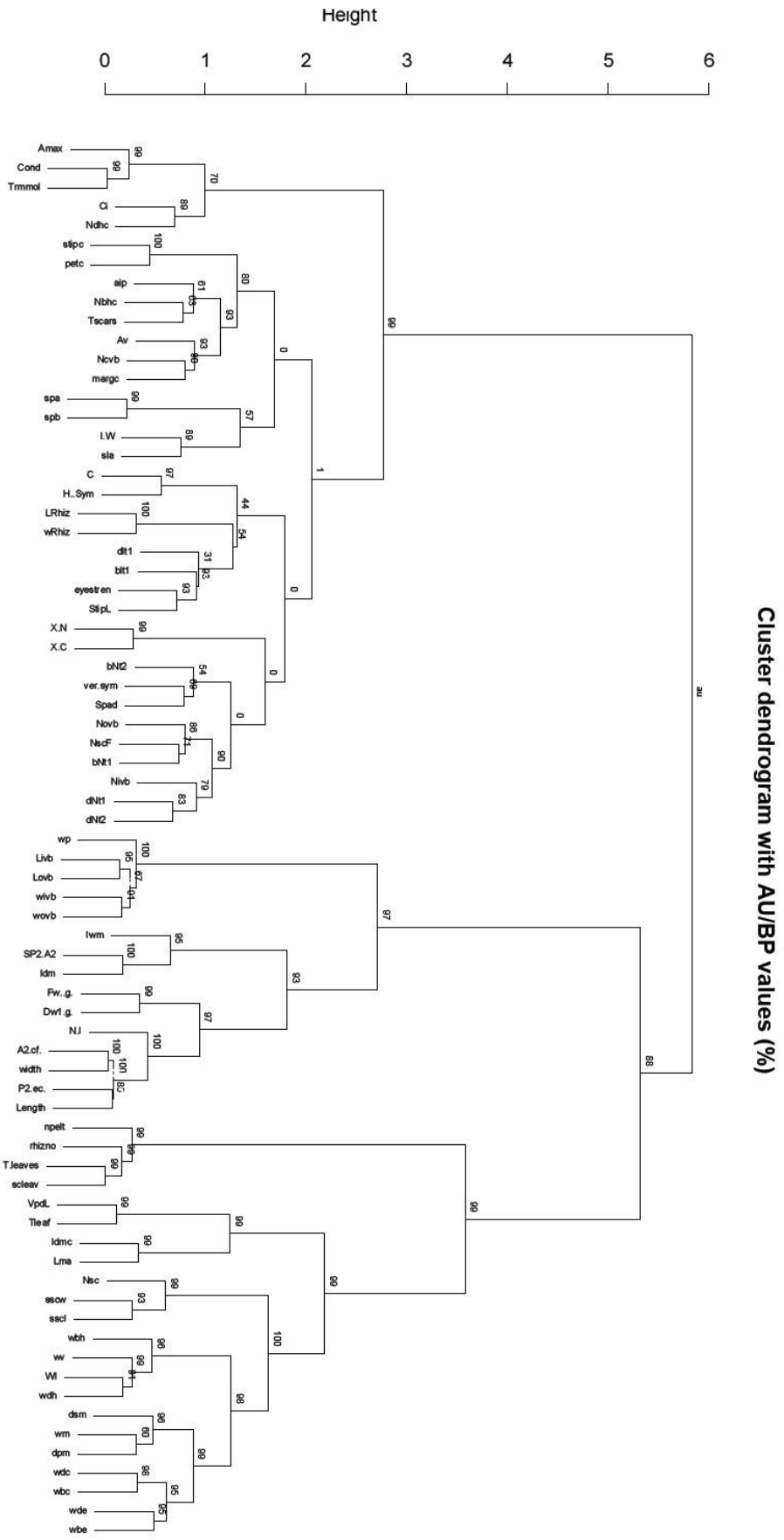

Figure 6.5: Cluster analysis of leaf phenotypic traits generated using correlation distance method (ward's method). The number on the nodes represents the support values based on 10,000 bootstrap replicas.

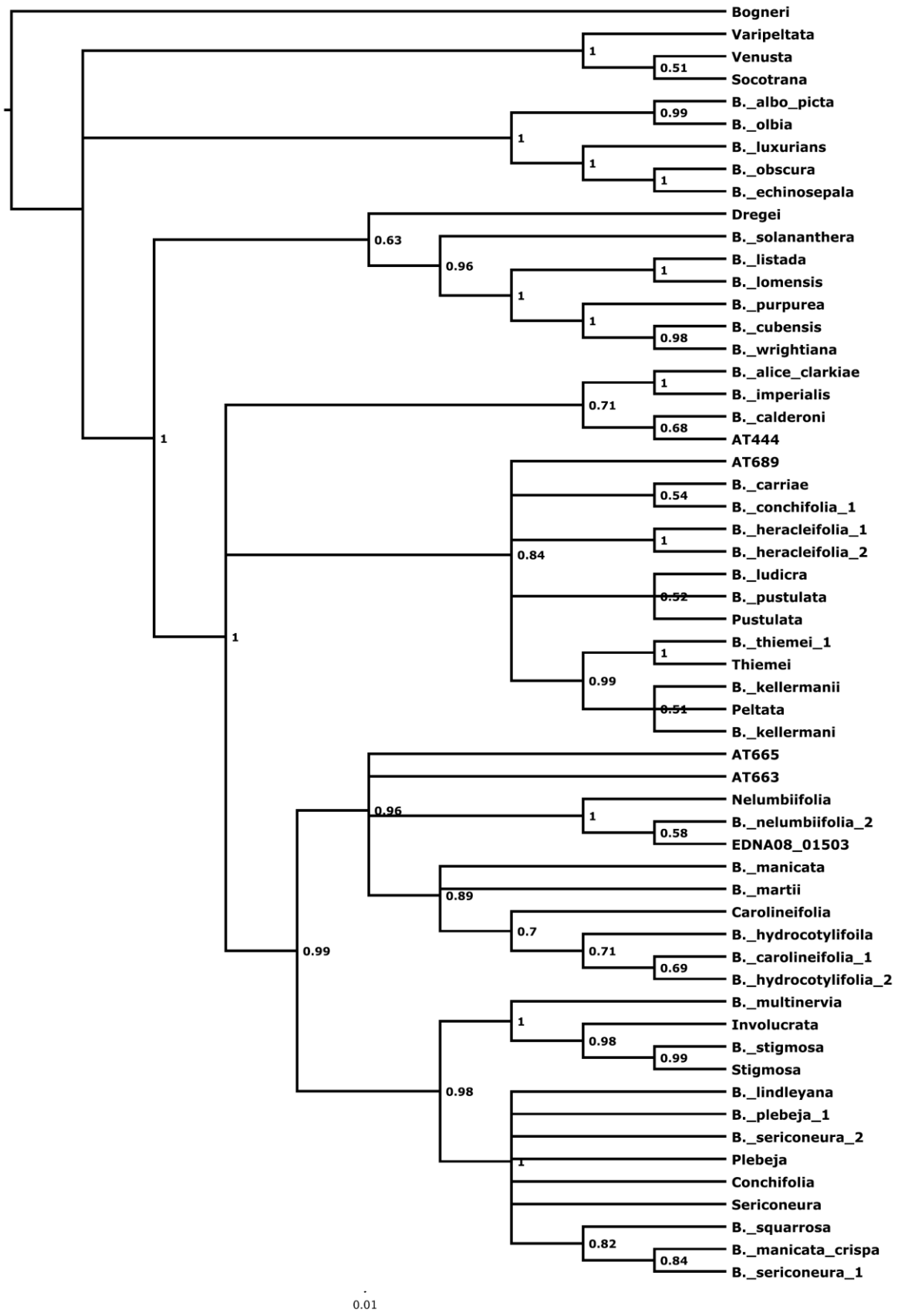

Figure 6.6: Bayesian Analysis of concatenated Region 21 and Region 24 complete sequences.

6.5 Multivariate Analyses confirmed pairwise patterns

Pairwise trait correlations gave useful information on the strength of association and the directionality of the traits. To extract further useful information from the data set a principal component analysis based on correlations was carried out on the variation in all the traits for the species in the section and the individuals in the mapping population.

Overall the first three principal components explained 33% of the variation in the combined data set. This is a large amount given the number of traits analysed (66). The first principal axis extracted 14% of the variation in the combined dataset. The first principal axis was most strongly influenced by traits related to leaf vascularisation patterning and mesophyll attributes and was less variable for leaf functional and general plant morphological traits (PCA1) (Figure 6.7). The second axis explained a further 12.07 % variation and was strong influenced by leaf size and shape traits. The third axis explained a further 7 % variation and was strong influenced by morphological traits (Table 6.1).

The distribution of the species on the principal axis yielded three clusters, one with large leaf areas including compound leaves such as *B. carolinefolia*, one with moderate leaf areas, such as *B. plebeja* and one with low leaf areas and high leaf mass areas such as *B. conchifolia*. A total of 15 studied species in the section Gireoudia belong to the second group i.e they produced moderate leaf areas. The principal component analysis clearly separated the species from the mapping population suggesting that the mapping population is transgressive and represented much of the variation seen in the section as a whole (Figure 6.7).

The variance of the eigenvectors observed in a principal components analysis conducted on the species, mapping population and their combined data set also confirmed trait- trait correlations.

Table 6.1: Principal components analyses of the trait data

	species		Mapping Population		Both sets	
PC	**Eigenvalue**	**% variance**	**Eigenvalue**	**% variance**	**Eigenvalue**	**% variance**
1	15.9	24.1	11.1	16.6	9.5	14.4
2	7.2	10.9	6.3	9.5	7.9	12.0
3	6.1	9.3	4.6	6.8	4.7	7.2
4	5.21	7.9	3.5	5.2	3.5	5.4
5	4.61	6.9	3.2	4.8	2.9	4.49

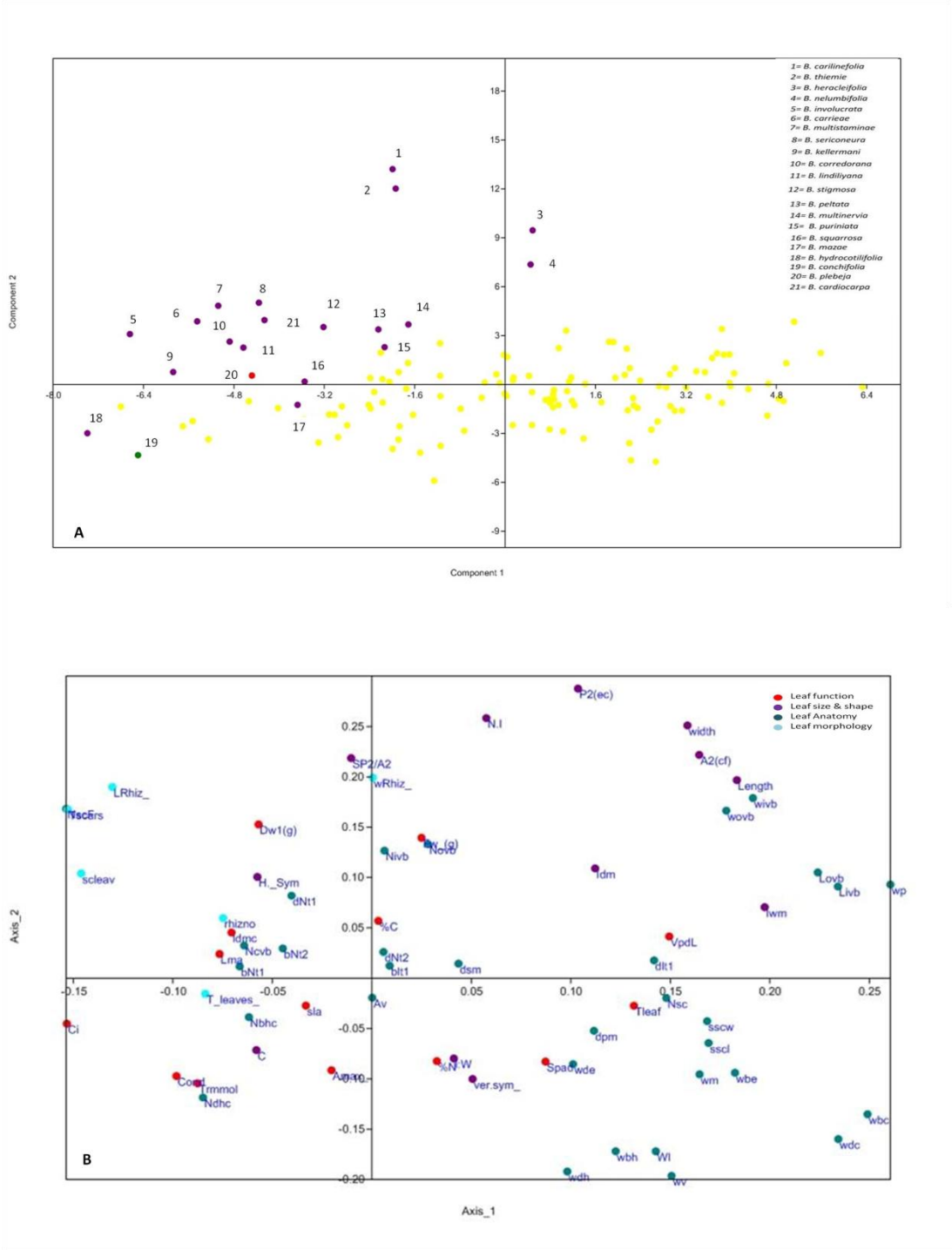

Figure 6.7: Principal component analysis of the studied species and mapping population ordinated according to the suite of leaf functional, size & shape, and micromorphological traits (A). Projection of the species and (A) variables on the principal component planes (B) are shown. Coloured circles denote samples belonging to the three groups: species (purple circles), *B. plebeja* (Red circle) and *B. conchifolia* (green) and mapping population (yellow).

6.6 Discussion:

There were many significant pairwise correlations between traits in the species correlation matrix. The correlations revealed interesting patterns that existed at the sectional level in the *Begonia* section Gireoudia. Some of the inter trait correlations observed in section Gireoudia presented apparently straightforward biological relationships and have been well documented as discussed in the results section of this chapter. However, we couldn't identify clear cut adaptive suites in this study. Traits generally found to be linked such as high A_{max} with high N_{mass} as well as high A_{max} with low LMA were not found across species in section Gireoudia. The absence of the correlation patterns observed globally among ecological leaf traits could be attributed to the small data set used here as well as the small range of variation observed for the traits.

Several significant trait-trait correlations were also observed in the mapping population and suggested genetic linkages between the traits. Whether or not the traits in the mapping population are genetically linked is investigated with the help of QTL mapping in chapter 7.

Cluster analysis classified *Begonia* species into two main groups. Comparisons of molecular phylogenetic tree of Central American *Begonia* species (Nichola Harrison unpublished) and the phenotypic cluster tree revealed evidence that closely related species have undergone phenotypic divergence in order to adapt to their respective habitats.

Results obtained from the principal component analysis more or less confirmed the grouping obtained by cluster analysis. Variation in all input variables was adequately explained by 5 principal components. Principal component analysis also indicated that there were several multivariate directions of variation in the suite of traits among the species and the mapping population investigated. It suggests that selection has acted on a number of traits to help them

adjust to their habitats. Principal component analysis also confirmed the transgressive nature of the traits in the mapping population.

CHAPTER 7. Quantitative trait loci (QTL) analysis

7.1 Brief overview:

The relative importance of major and minor genetic changes in evolution has been a subject of intense debate. Fisher argued that the process of adaptation takes place through the accumulation of many beneficial mutations of small effect. He analysed the genetics of adaptation through his ‘‘geometric model’’ and came to the conclusion that the chance a mutation will be adaptive is nearly 50% for mutations of small effects and approximately zero for mutations of large effects (Fisher, 1930; Orr, 2005a).

Motoo Kimura (1983) challenged Fisher’s findings and suggested that relative to major effect mutations, minor effect mutations may be more likely to be beneficial, but are less likely to go to fixation. He supported intermediate effect mutations as the most likely mutations to underlie adaptations. Orr (1998a) proposed that mutations of large effects have a higher chance of getting fixed earlier and are followed by mutations of decreasing effects as the population progresses towards its fitness optimum.

The most reliable data on the architecture of natural variation between species comes from Quantitative Trait Locus (QTL) analysis (Orr, 1998). QTL mapping uses segregation of traits and genotypes in segregating populations that may be made from lab strains (Curley et al., 2005), wild accessions (Marri et al., 2005), or segregate in the wild (Maria et al., 2012). The segregating populations are genotyped at loci covering the whole genome and phenotyped for the traits of interest to find associations between loci and phenotype. Several types of markers could be used to carry out QTL mapping including single nucleotide polymorphic markers,

microsatellites, and amplified fragment length polymorphic markers, but the denser the map the more reliable the analysis (Casa et al., 2000).

QTL analysis has helped evolutionary biologists understand a number of issues including the genetic basis of crop domestication. Work carried out on the genetics of domestication in important crop plants has so far revealed that in many cases a small number of changes in major genes are primarily responsible for transition from the wild form into a domesticated form (Sang, 2009, Gross and Olsen 2010).

The first domestication gene cloned was from tomato. Tanksley and coworkers (1980) initiated QTL analysis in a cross between wild and domesticated tomato and identified a region harbouring the major QTL fruit weight 2.2 (*fw2.2*). Transgenic studies confirmed the phenotypic effect of *f.w2.2* (Tanksley 1988; Tanksley, 2000). Orthologues of this fruit weight QTL have also been mapped in eggplant and pepper suggesting an underlying common aspect of plant development and evolution (Ben Chaim et al., 2001). Domestication genes have also been cloned in maize rice, and barley (Doebley et al., 2006; Li et al., 2006b; Komatsuda et al., 2007).

Doebley and coworkers investigated differences in plant architecture between maize and its ancestor teosinte. Mapping and mutation analysis studies resulted in the isolation of many of the genes responsible for phenotypic differences between maize and teosinte. These included *teosinte branched1* (*tb1*) and *teosinte glume architecture* (*tga*). Both the genes are involved in controlling lateral branching, a trait that contributes to differences in inflorescence and vegetative architecture (Doebley, 1995). This has been confirmed by phenotypes of the null mutations in maize. In addition to these two remarkable examples, cloning of QTLs in argonomically important crops such as maize, rice, wheat, barley, and tomato have also led to

the identification of several other genes selected during domestication (Doebley et al., 1997; Frary et al., 2000; Wang et al., 2005 and Komatsuda et al., 2007).

QTL analysis of natural variation

Quantitative trait loci (QTL) mapping has also been used to study the genetic basis of natural variation in morphological, physiological and anatomical traits by mapping QTLs underlying differences between populations and species (Mackay, 2001a, b; Mauricio, 2001; Rieseberg et al., 2002, 2003).

Genetics underlying morphological traits:

A number of genes known to affect variation in general morphological traits have been identified by using the natural variation in *Arabidopsis thaliana, S. lycopersicum, O. sativa* and Maize. These include genes and loci that influence flowering e.g., *FR1* (Johanson et al., 2000), seedling growth e.g., *PHYA* (Maloof et al., 2001), root growth e.g., *BRX* (Mouchel et al., 2004), and *LPR1* (Svistoonoff et al., 2007) in Arabidopsis, fruit shape e.g., *SUN* in *S. lycopersicum* (Xiao et al., 2008), germination e.g., *QLTG3*, (Fujino et al., 2008), and seed size e,g., *GW2* in Rice (Song et al.,2007) and inflorescence architecture e,g., *TB 1* in Maize (Doebley et al., 1997).

Quantitative trait locus analysis has provided ample evidence that the variation within and between species for many traits are controlled by relatively few genes of major effect. "*Shattering4* (*sh4*)" and "*Fruitweight2.2* (*fw2.2*)" were identified as major effect QTLs responsible for seed shattering in wild rice and fruit mass in tomato respectively (Frary et al., 2000). Similarly, *grain number1* (*gn1*) was also identified as a major effect QTL that controlled grain number differences between rice varieties (Ashikari et al., 2005).

Other traits such as root and floral morphology have also helped evolutionary biologists for unveiling the genetic architecture of morphological traits. A Quantitative trait locus analysis of a cross between accessions of *Arabidopsis* revealed a locus that controlled difference in root length. The locus explained 80 % of the variation in the root length. In a study of floral morphology in *A. thaliana*, 18 QTLs were found, eleven of which were associated with more than one floral trait signifying tight morphological integration of the flower (Juenger et al., 2000). This illustrates the constraints that operate on adaptation for such traits.

Genetics underlying leaf size and shape:

To date a large number of genes known to affect variation in leaf size and shape have been identified (Tsukaya, 2005, Byrne, 2012). The multifactorial nature of the observed natural variations in leaf architecture has also been confirmed by Quantitative Trait Loci (QTL) mapping. Micol and colleagues analyzed variations in the architecture of vegetative leaves in *Arabidopsis* accessions and identified more than 20 QTLs, harboring naturally occurring alleles that contribute to natural leaf size variations (Pérez-Pérez et al., 2002). Similar results were also seen by Andrew Hudson and colleagues in a hybrid population between *A. majus* that possess large leaves and *A. molle* that possess small leaves suggesting the complex genetic architecture of leaf size traits (Feng et al., 2009). Yue and colleagues also resolved a total of 17 QTLs for morphological traits (flag leaf length, width, and area), using interfertile hybrids between indica (*O. sativa* L. ssp. indica) cultivar and japonica (*O. sativa* L. ssp. japonica) cultivars. They confirmed that overall leaf size or length and width are independently controlled by several loci (Yue-B et al., 2006).

Genetics underlying physiological traits:

QTL mapping studies on physiological traits in crop plants has helped us understand that a few loci of major effect can be responsible for the evolution of these ecologically important traits (Teng et al., 2004; Zhao et al., 2008; Adachi et al., 2011).

Four QTLs in maize and three QTLs in *Helianthus* were identified for net assimilation rate and together explained 34.37% and 62.9% of the total phenotypic variation respectively (Gentzbittel et al., 2001). The genetic architecture of chlorophyll content revealed 13 QTL in Rice and 4 QTL in *Helianthus* explaining 56.19% and 53 % of the variation in the trait. Four putative QTL were also detected in barley for chlorophyll content that explained 38 % of the total phenotypic variation (Xue et al., 2008).

The genetic architecture of physiological traits in *Helianthus* also revealed four chromosomal regions associated with stomatal conductance that explained 61.9 % of the total variance. A total of three QTL for transpiration, one QTL for internal CO_2 concentration and two loci for nitrogen content per leaf area were resolved in an inbred population between two *Helianthus* annus plants (Gentzbittel et al., 2001; Riesberg et al., 2007).

Other physiological traits such as those related to resource use strategy such as leaf carbon content and specific leaf area have also been studied. For leaf carbon content, two epistatic loci explained 2 percent of the variation in *Helianthus* (Riesberg, 2007). A QTL for SLA mapped in *Aegilops tauschii*, the D-genome donor of hexaploid wheat explained 8% of the variation in the trait (Peters et al., 2005).

Genetics underlying micromorphological traits:

To date a number of genes known to affect internal leaf anatomy have been identified including genes that influence the polar elongation of cells e.g., *Angustifolia* and *Rotundifolia3* and genes that affect both the division and elongation of cells e.g., *curly leaf* (Byrne, 2012, Tsuge et al., 1996; Kim et al., 1998; Tsukaya and Uchimiya, 1997).

Many complex traits such as stomatal patterning are unlikely to be controlled by a single gene (Peterson et al., 2010, Bradshaw and Stettler 1995, Wu et al., 1997). This has been confirmed by mutational analysis that has identified a number of genes known to affect stomatal pattern formation e.g. too many mouths (*tmm*), and stomatal density and distribution1 (*sdd1*) (Dong and Bergmen, 2010). Loss-of-function *tmm* mutants have an increased stomatal density as well as clustered stomata phenotype. On the other hand, mutants with defects in the *SDD1* locus show increased stomatal density (Yang and Sack, 1995). Triple mutants of the *ERECTA* gene family *er, erl1, erl2* members also results in severe stomatal clustering (Casson1 and Gray, 2007).

Mutation analysis has also identified several genes implicated in the initiation and development of trichomes including glabra1 (*gl1*), transparent testa glabra (*TTG*), and Loss-of-function (*gl3)* (Grebe, 2012; Galway, 1994; Koornneef, 1981; Hulskamp et al, 1994). The null alleles of these genes resulted in plants with no or very few trichomes. Genes that regulate trichome density have also been identified via quantitative genetics analysis. The reduced trichome number (*RTN*) gene was discovered as a quantitative trait locus variant between the Columbia and Landsberg erecta ecotypes of *Arabidopsis* (Larkin, 1996).

Work carried out to explore the genetics of traits associated with vascularisation patterning has also revealed quite a few mutants with altered leaf vascular patterning (Cano-Delgado et

al., 2010) and stem vascular patterning (Sanchez et al., 2012). For example the *Arabidopsis monopteros* mutants are characterised by missing and interrupted leaf marginal veins, where as the *loppedl* mutants are characterised by leaves that are narrowed with a bifurcated and twisted midvein (Carland and McHale, 1996; Przemeck et al., 1996). Midribless mutants have also been recovered from maize, barley, millet, and other grasses (Seip and Tsuchiya, 1979; Rao et al., 1989; Fladung et al., 1991; Fladung, 1994). Narrow-leaf mutants have also been described in maize, *Antirrhinum*, tobacco, and other species. These mutants are shown to approach radial symmetry. Moreover their vascular system is reduced to a single midvein with secondary veins ranging from zero to few (McHale, 1992; Waites and Hudson, 1995).

QTL mapping carried out on traits related to vascularisation patterning has resolved QTLs of major effect. A total of three QTLs that explained 58.8% of the total variations were detected for number of vascular bundles in a cross between an *indica* cultivar and a *japonica* cultivar. Among them, the largest effect individually accounted for 31.1% of the total variation (Zhang et al. 2002).

Genetics of speciation:

QTL analysis has been used extensively to identify the principal genes involved in the process of speciation (Lexer and Widmer, 2008). Toby Bradshaw, Douglas Schemske and their colleagues (1998) studied speciation in monkeyflowers (*Mimulus*). Two species with contrasting floral traits and different pollinator preferences were subjected to QTL mapping. QTLs were identified for a number of floral traits and served as possible candidates for 'speciation' genes due to the reinforcement effects they had on reproductive isolation. The QTLs identified had a major effect on the floral traits i.e they accounted for more than 25% of the phenotypic variance in floral morphology, leading to the conclusion that the evolution

of reproductive isolation between the species involved genes of major effect (Bradshaw, 1995).

Evidence for the involvement of major effect genes controlling reproductive isolation also came from a study conducted on the natural hybrids of Louisiana irises. QTL mapping analysis carried out on floral traits in populations of hybrids between *Iris fulva* and *Iris brevicaulis* revealed QTLs of large effects which tended to colocalize suggesting that genetic linkage of floral and colour traits might have an integral role in facilitating the maintenance of phenotypic divergence (Arnold et al., 2008).

QTL analysis has also been used to identify speciation genes that could be the key targets of habitat specific natural selection. Work carried out on serpentine soils provides strong evidence for major effect genes underlying habitat adaptation and the evolution of plant ecotypes (Bratteler, 2006). Bratteler and colleagues were able to identify twenty four major QTLs for twelve morphological, physiological and life-history traits from an F2 population derived from and intraspecific cross between serpentine and non serpentine ecotypes of *Silene vulgaris*. Major QTLs had a percentage of variance over 25%. This suggested that traits potentially involved in adaptation to serpentine soils are controlled by few genes of major effect (Bratteler, 2006).

7.2 Objectives:

This chapter examines the genetic architecture of morphological, ecophysiological and micromorphological traits in an F1 backcross population between two *Begonia* species, *B. plebeja* and *B. conchifolia,* found in two different habitats in Central America. The cloning of domestication genes such as *tb1* in maize (Doebly et al., 1997) and QTL mapping studies discussed in the introduction of this chapter has provided enough evidence for the role of

major effect genes in plant adaptation. Based on this my main goal of this study was to test the hypothesis that genes of major effect underlie species-level variation in *Begonia* section Gireoudia.

7.3 Results:

To explore the genetics of morphological, physiological, and anatomical traits in *Begonia* section Gireoudia, QTL analysis was carried out. For the categorical data (binary traits) a series of contingency tables and chi-squared tests for independence of (e.g, colour scores) were tabulated against the genotypes at each marker in turn, with markers in map order. The significance of the chi-squared statistic against the marker location was plotted to identify the genome locations associated with trait differences. This was carried out in R software using the following code (Appendix 7.3) (http://www.r-project.org).

Categorical traits having more than two categories (4 categories) were analysed by using a non parametric non-parametric method MIM-GLZ based on the Generalized Linear Model (GLZ) in Qgene (Nelson, 1997).

Mapping of count data and continuous traits was done with composite interval mapping first on untransformed data in Qgene. Genome-wide threshold LOD values to declare a QTL to be significant were determined using 1000 permutations in Qgene. The analysis resolved a number of QTLs. To confirm whether transforming the data would pull out further QTLs, transformations were carried out. The two kinds of transformations used were log and square root transformations. Square root transformations were used on count data (e.g, number of trichomes). Log transformations were used for continuous traits such as total leaf area. Trait transformations did not resolve any further QTLs for the traits.

Traits that were skewed and did not achieve normality after transformation procedures were analysed using a non-parametric method MIM-GLZ based on the Generalized Linear Model (GLZ) in Qgene. This plug in Qgene can handle binary, ordinal, count and continuous traits and specializes in treating non-normal data. The analysis resolved three QTLs that were not resolved by the first two data sets i.e the untransformed and the transformed data set.

Given below is the detailed description of the QTL analysis carried out for different categories of traits. Locations of significant QTLs on the 14 chromosomes resolved in this study are shown in Figure 7:65.

7.3.1. Morphological traits:

a) Estimation of QTL positions by chi- squared tests for independence (Binary traits):

For the chi-squared tests for independence analysis of binary morphological traits in our mapping population, 162 markers covering the whole genome were used as background markers. The chi-squared tests for independence of trait scores tabulated against the genotypes at each marker in turn resolved QTLs for two binary traits including presence/absence of blotches and presence /absence of a red petiole base.

Two markers RCAA_82 (chrm 9 21.8cM) and TFC13 (chrm 9 22.6cM) were significantly associated with presence / absence of blotches, confirming the presence of a controlling locus for this trait around this position.

A region having four markers including GCAG_163, BCLV2, RCAG_194b and BMYBTF3 present on chromosome 14 was significantly associated with presence/absence of red petiole base. These markers spanned an interval of 36 cM to 39.8 cM on the chromosome. BCLV2

and BMYBTF3 are both sequenced markers but neither gene family is involved in the regulation of anthocyanin.

b) Estimation of QTL positions by MIM_GLZ (ordinal traits with up to four categories):

The MIM_GLZ analysis option was used in Qgene for the analysis of petiole colour. The analysis identified a QTL for petiole colour on linkage group 14 at a position of 36 cM and had a positive additive effect.

c) Estimation of QTL positions for continuous traits by simplified CIM:

For the analysis of continuous traits, the simplified CIM analysis option was used in Qgene. Results are presented in figure 7.1 to 7.7 of this chapter. Permutation tests with 1000 random repeats failed to establish any of the LOD peaks as significant.

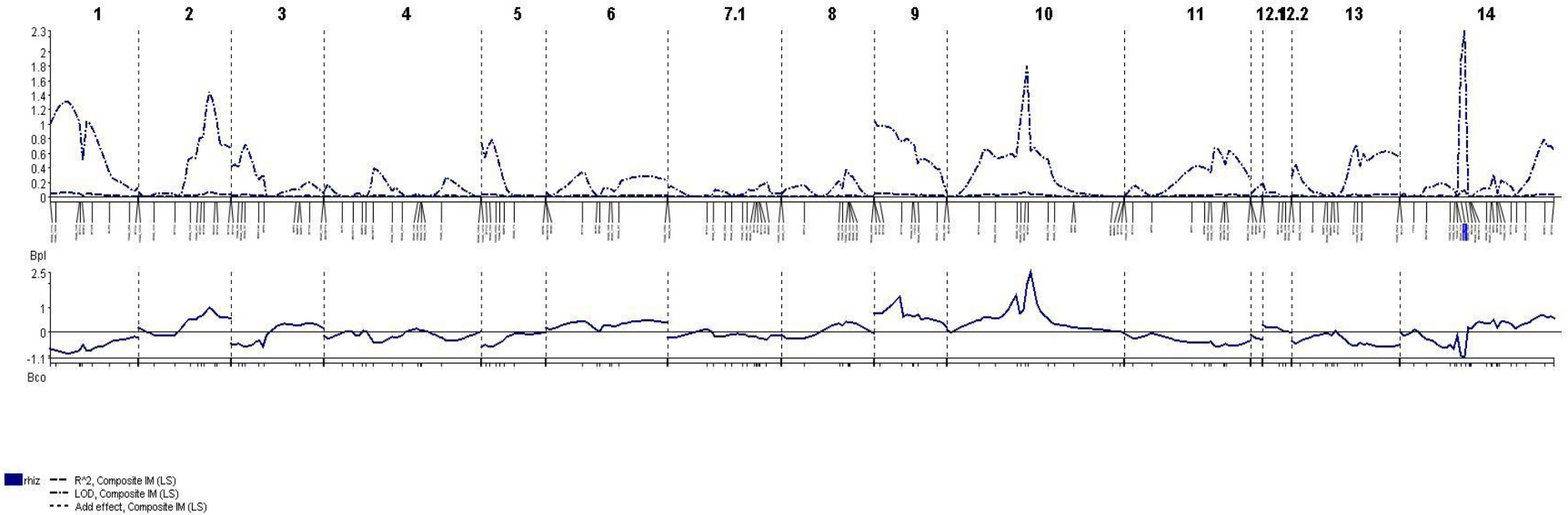

Figure 7.1: Rhizome numbers scan of a test statistic for sCIM. A total of 162 markers covering the whole genome were used as background markers. LOD support intervals were calculated from the CIM results. Genome-wide threshold LOD values to declare a QTL to be significant were determined with 500 permutations. The threshold values obtained with 500 permutations were alpha 0.10 = 2.954, alpha 0.05 = 3.342 and alpha 0.01 = 4.479. Default measurements were used in QGENE to select cofactors. Selected background locus included GCAG_184 14 34.5.

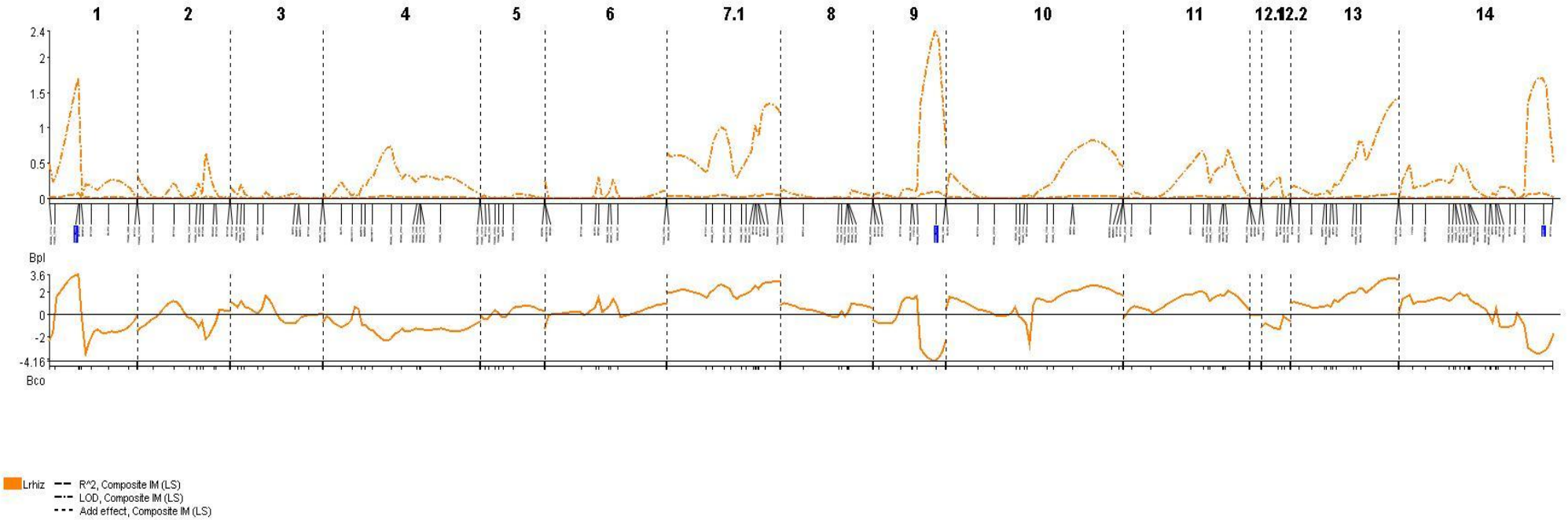

Figure 7.2: Length of rhizome scan of a test statistic for sCIM. A total of 162 markers covering the whole genome were used as background markers. LOD support intervals were calculated from the CIM results. Genome-wide threshold LOD values to declare a QTL to be significant were determined with 500 permutations. The threshold values obtained with 500 permutations were alpha 0.10 = 2.8, alpha 0.05 = 3.765 and alpha 0.01 = 5.2. Default measurements were used in QGENE to select cofactors. Selected background locus included YCAA_120 1 18.0, GCAC_174 9 35.8, BERF1 14 81.8.

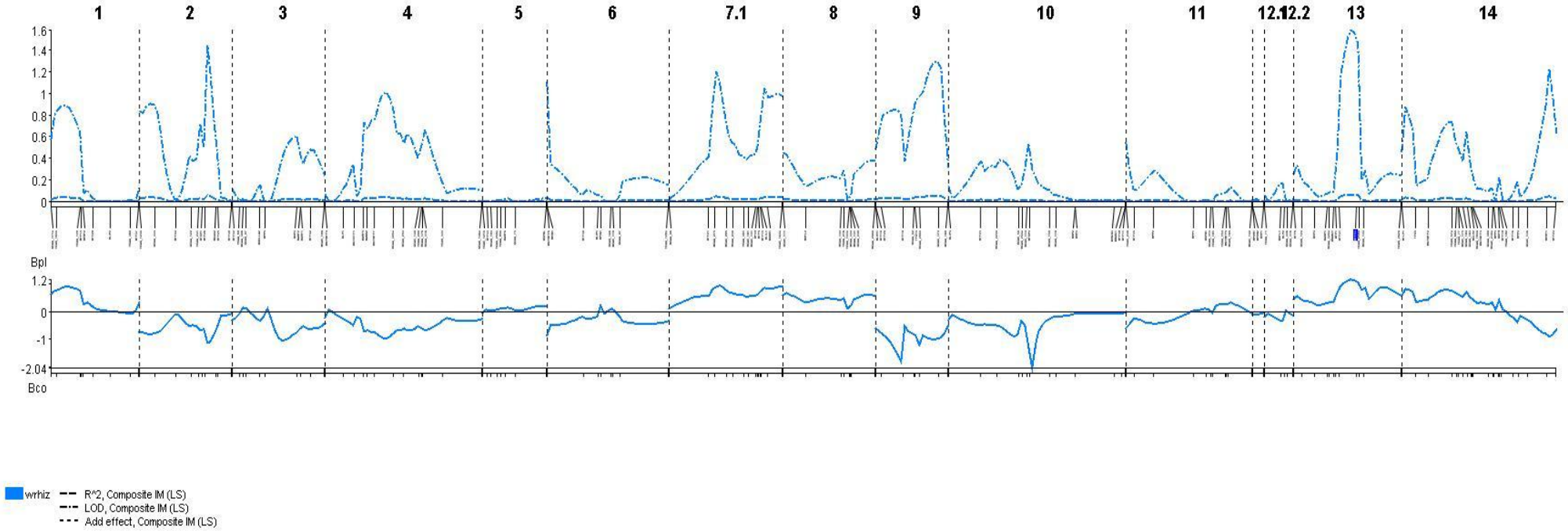

Figure 7.3: Width of rhizome scan of a test statistic for sCIM. A total of 162 markers covering the whole genome were used as background markers. LOD support intervals were calculated from the CIM results. Genome-wide threshold LOD values to declare a QTL to be significant were determined with 500 permutations. The threshold values obtained with 500 permutations were alpha 0.10 = 2.613, alpha 0.05 = 3.244 and alpha 0.01 = 5.186. Default measurements were used in QGENE to select cofactors. Selected background locus included BTF43 13 51.8.

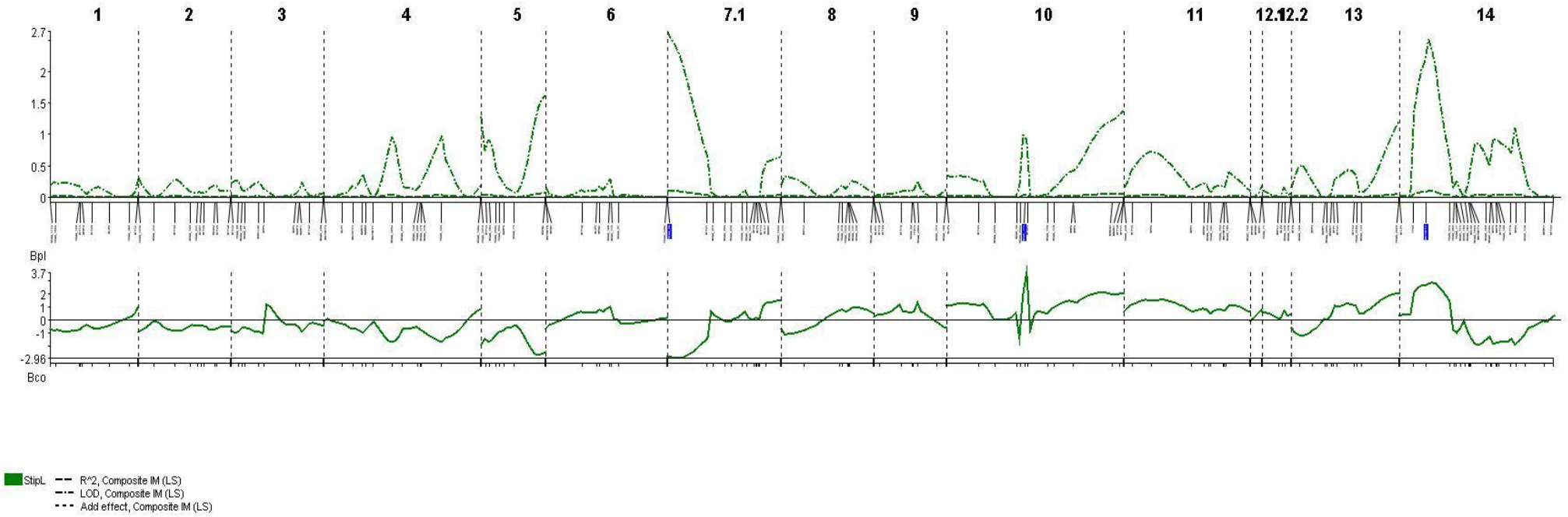

Figure 7:4: Stipule length scan of a test statistic for sCIM. A total of 162 markers covering the whole genome were used as background markers. LOD support intervals were calculated from the CIM results. Genome-wide threshold LOD values to declare a QTL to be significant were determined with 500 permutations. The threshold values obtained with 500 permutations were alpha 0.10 = 2.873, alpha 0.05 = 3.281 and alpha 0.01 = 4.562. Default measurements were used in QGENE to select cofactors. Selected background locus included RCAA_86 7.1 0.0, BCAC_114 10 44.2, BMYBTF2 14 15.1.

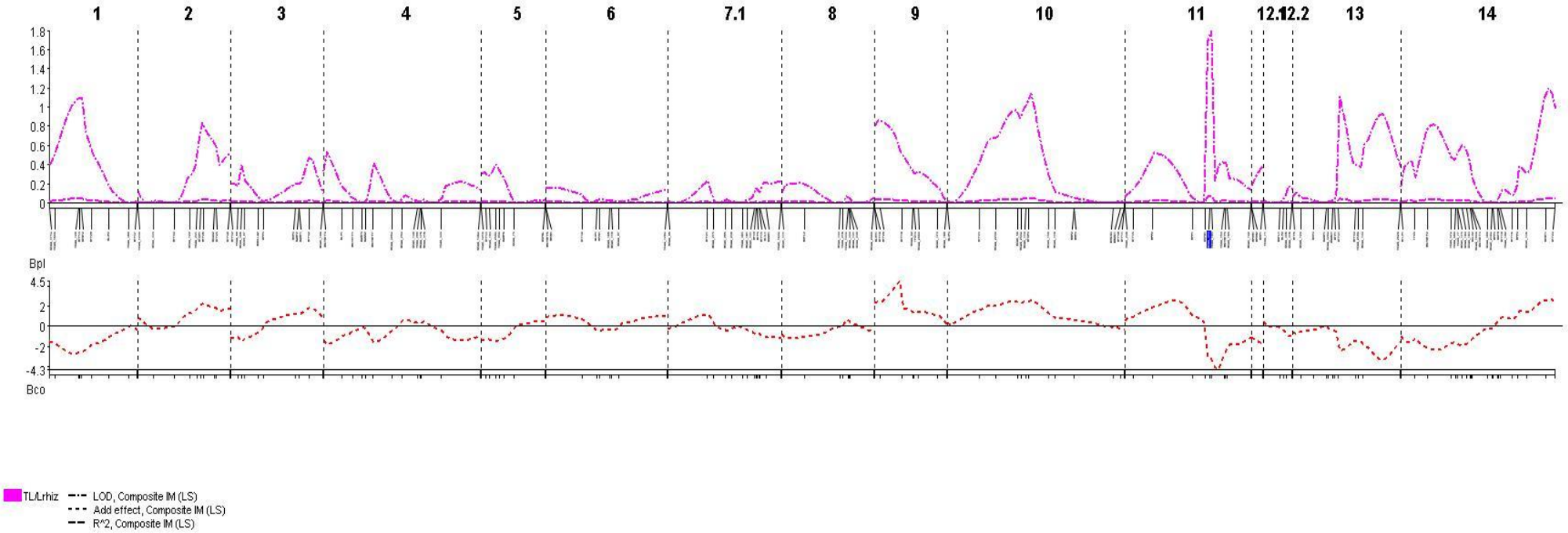

Figure 7.5: Number of leaves per cm length of rhizome scan test statistic for sCIM. A total of 162 markers covering the whole genome were used as background markers. LOD support intervals were calculated from the CIM results. Genome-wide threshold LOD values to declare a QTL to be significant were determined with 500 permutations. The threshold values obtained with 500 permutations were alpha 0.10 = 2.976, alpha 0.05 = 3.505 and alpha 0.01 = 4.493. Default measurements were used in QGENE to select cofactors. Selected background locus included RCAG_254a 10 41.7.

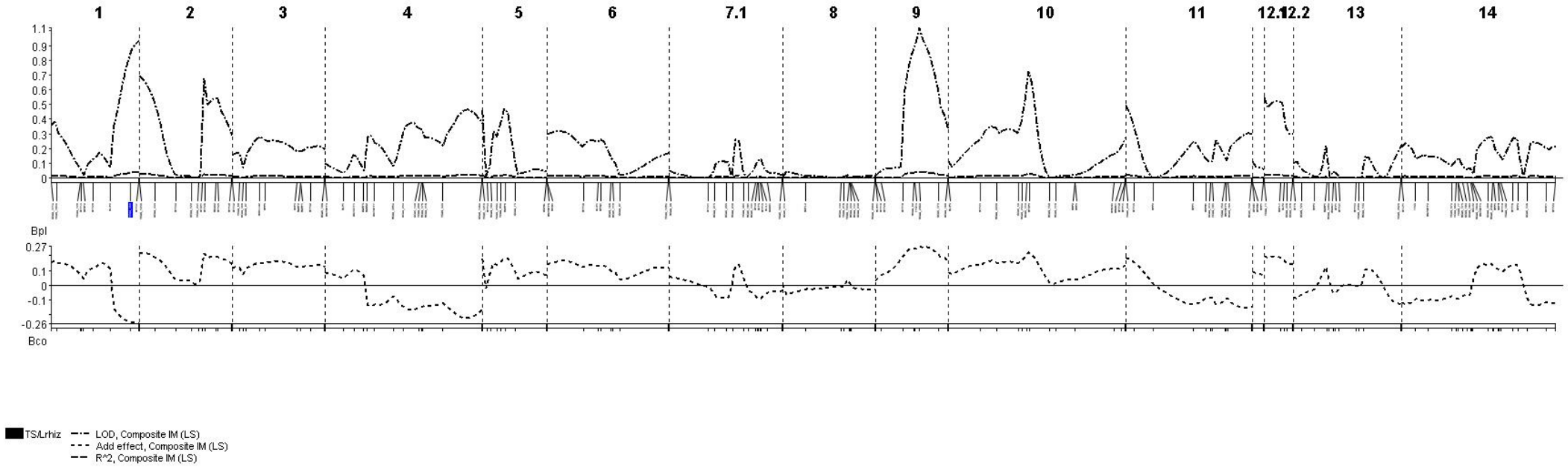

Figure 7.6: Total scars per cm length of rhizome scan of a test statistic for sCIM. A total of 162 markers covering the whole genome were used as background markers. LOD support intervals were calculated from the CIM results. Genome-wide threshold LOD values to declare a QTL to be significant were determined with 500 permutations. The threshold values obtained with 500 permutations were alpha 0.10 = 2.939, alpha 0.05 = 3.914 and alpha 0.01 = 5.617. Default measurements were used in QGENE to select cofactors. Selected background locus included RCAA_298b 9 25.2.

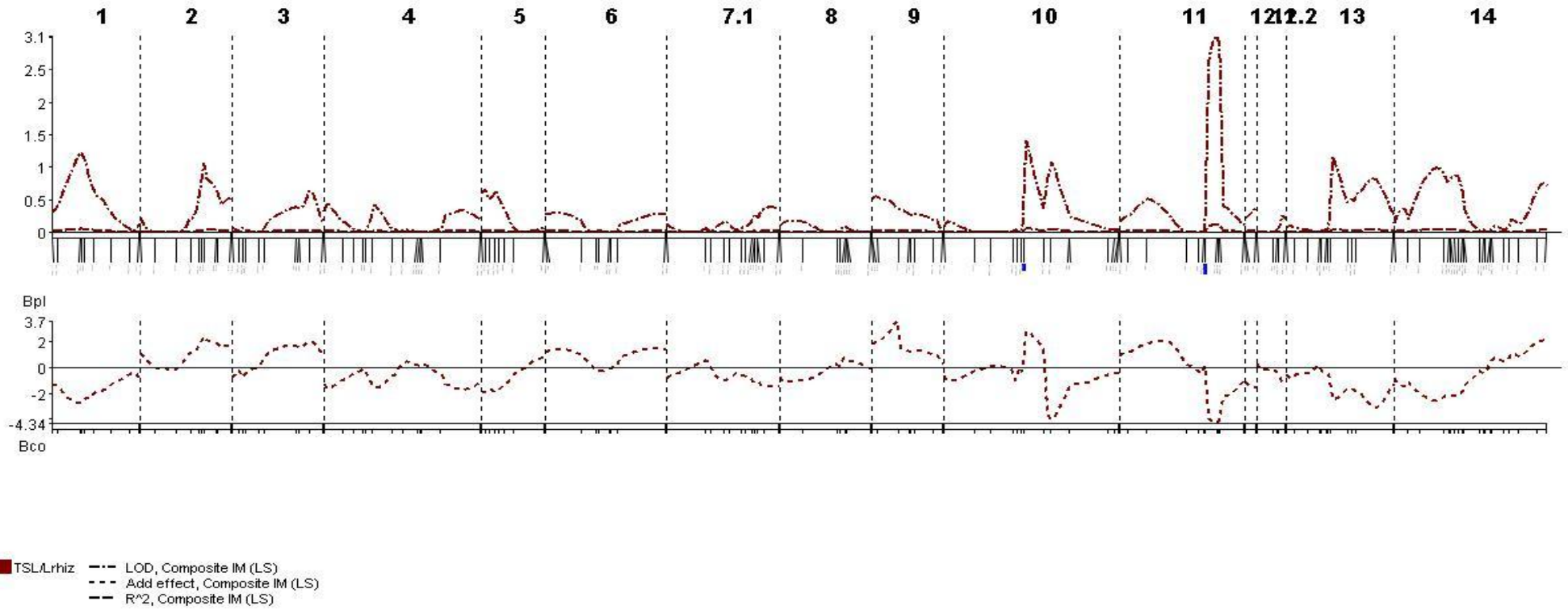

Figure 7.7: Total scars & leaves per cm rhizome scan of a test statistic for sCIM. A total of 162 markers covering the whole genome were used as background markers. LOD support intervals were calculated from the CIM results. Genome-wide threshold LOD values to declare a QTL to be significant were determined with 500 permutations. The threshold values obtained with 500 permutations were alpha 0.10 = 3.284, alpha 0.05 = 3.734 and alpha 0.01 = 4.593. Default measurements were used in QGENE to select cofactors. Selected background locus included RCAG_254a 10 41.7 and YCAG_57a 14 28.3.

7.3.2. Physiological traits:

a) Estimation of QTL positions by simplified CIM for physiological traits in *Begonia*:

No significant QTLs were found for net assimilation rate and stomatal conductance in our analysis. To explore this further, parameters measured or calculated during the measurement of net assimilation rate and stomatal conductance by LICOR-6400 such as intercellular CO_2 concentration (Ci), leaf transpiration (Trmmol) and vapor pressure deficit calculated from leaf temperature (VpdL), were subjected to QTL analysis. Interestingly the analysis resolved a significant QTL for vapour pressure deficit on chromosome 13 at an interval of 42cM and explained 16% of the total variation for this trait (Figure 7.12). The locus had a positive additive effect.

Significant QTLs were found for leaf chlorophyll content and leaf mass area. The QTL for leaf chlorophyll content was located on chromosome 8 at an interval of 18.7 - 28.7cM and explained 13% of the total variation for this trait (Figure 7.21). The locus had a positive additive effect. Other studies have also detected large effect QTLs for chlorophyll content. In *Helianthus* chlorophyll content was linked to 4 QTLs that together explained 53 % of the variation in the trait (Gentzbittel, 2001). Four putative QTLs have been reported for chlorophyll content in barley. Individual QTL explained from 6.3 to 20.2 % of the total phenotypic variation (Zhang et al., 2008). The QTL for chlorophyll content in this population is located close to a QTL for indent depth mean. However the two traits did not appear correlated in the spearman rank analysis (Table 7.2).

A QTL for total leaf mass area variation was indentified on chromosome 4 at an interval of 76 – 84 cM which explained 12.7% of the variation in the trait (Figure 7.18). The locus had negative additive effect.

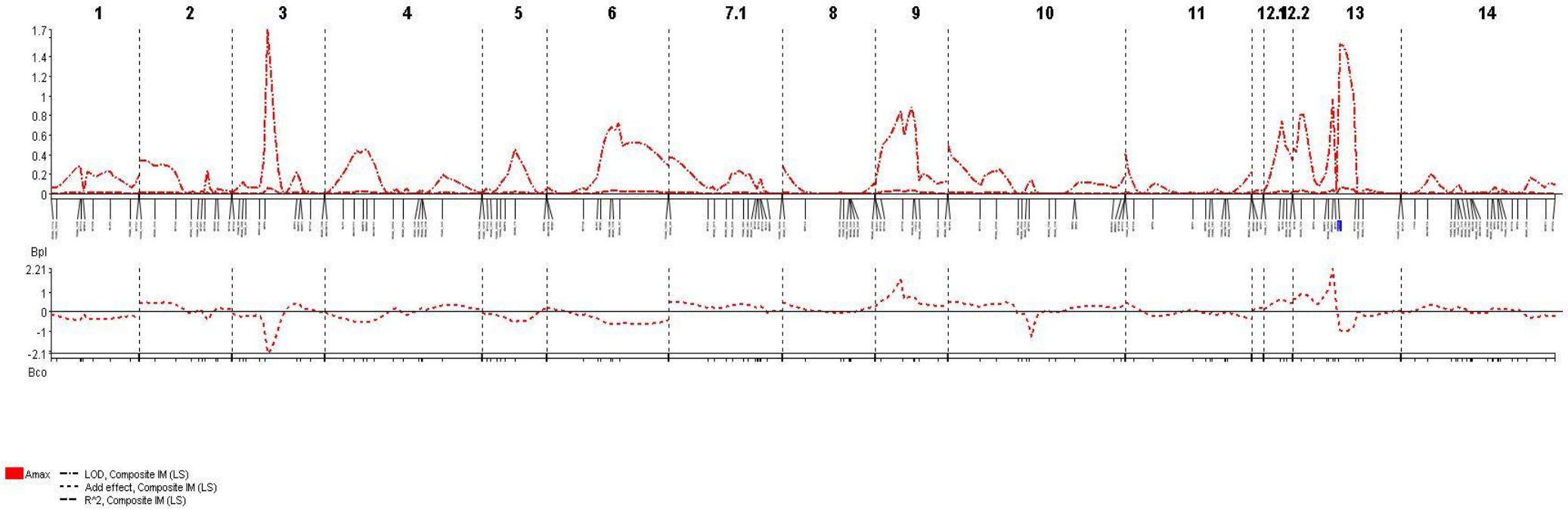

Figure 7.8: Net Assimilation rate scan of a test statistic for sCIM. A total of 162 markers covering the whole genome were used as background markers. LOD support intervals were calculated from the CIM results. LOD score along the linkage group with the threshold level is indicated by the dashed line. Genome-wide threshold LOD values to declare a QTL to be significant were determined with 500 permutations. The threshold values obtained with 500 permutations were alpha 0.10 = 2.824, alpha 0.05 = 3.367, and alpha 0.01 = 4.561. Default measurements were used in QGENE to select cofactors. Selected background locus included BTF41 13 41.9.

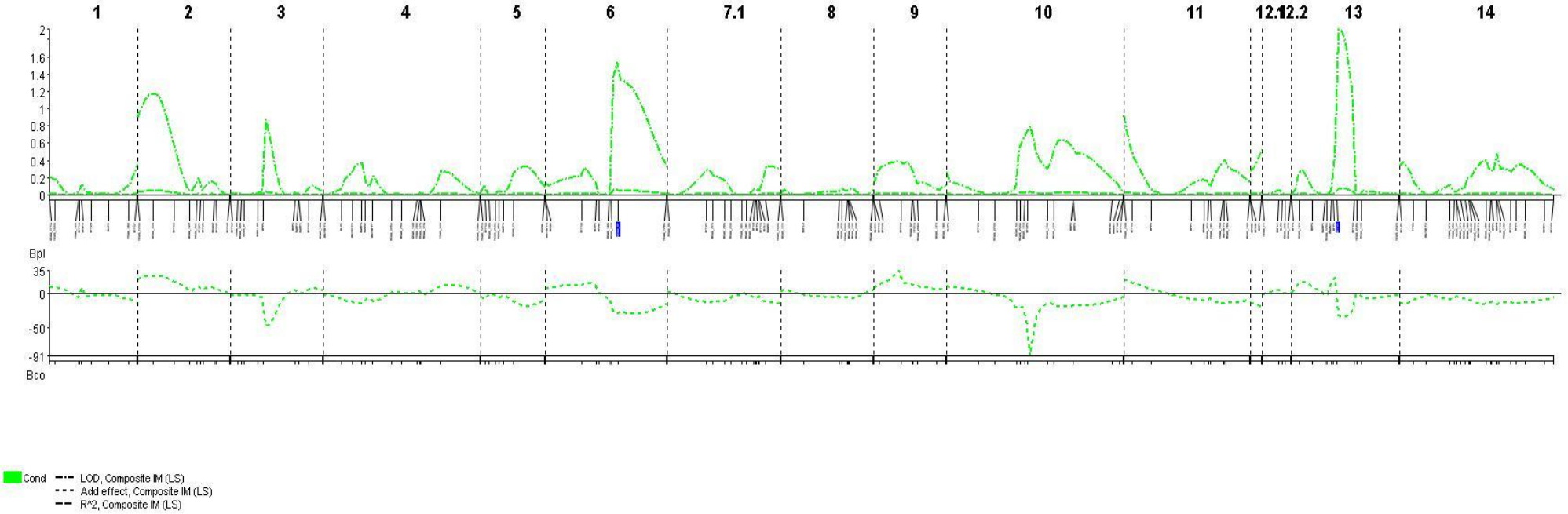

Figure 7.9: Stomatal conductance scan of a test statistic for sCIM. A total of 162 markers covering the whole genome were used as background markers. LOD support intervals were calculated from the CIM results. Genome-wide threshold LOD values to declare a QTL to be significant were determined with 500 permutations. The threshold values obtained with 500 permutations were alpha 0.10 = 2.665, alpha 0.05 = 3.201 and alpha 0.01 = 4.313. Default measurements were used in QGENE to select cofactors. Selected background locus included GCAA_97 6 41.5, BTF41 13 41.9.

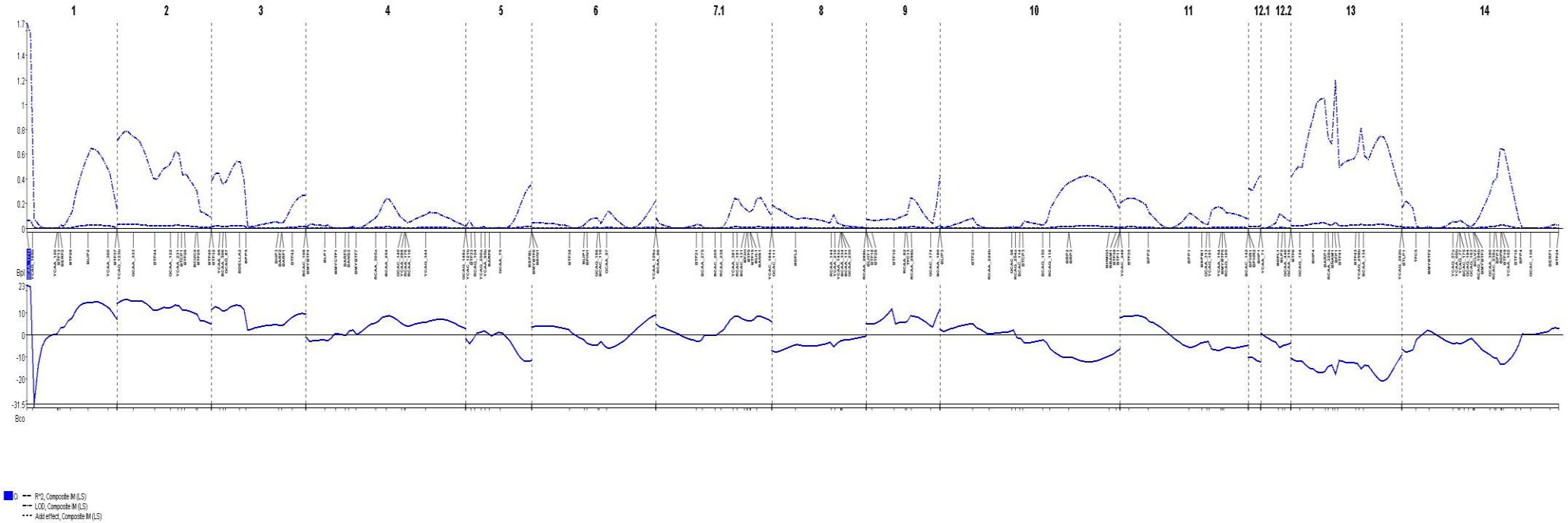

Figure 7.10: Internal carbondioxide scan of a test statistic for sCIM. A total of 162 markers covering the whole genome were used as background markers. LOD support intervals were calculated from the CIM results. Genome-wide threshold LOD values to declare a QTL to be significant were determined with 500 permutations. The threshold values obtained with 500 permutations were alpha 0.10 = 2.624, alpha 0.05 = 2.978 and alpha 0.01 = 4.041. Default measurements were used in QGENE to select cofactors. Selected background locus included GCAA_171a 1 1.2.

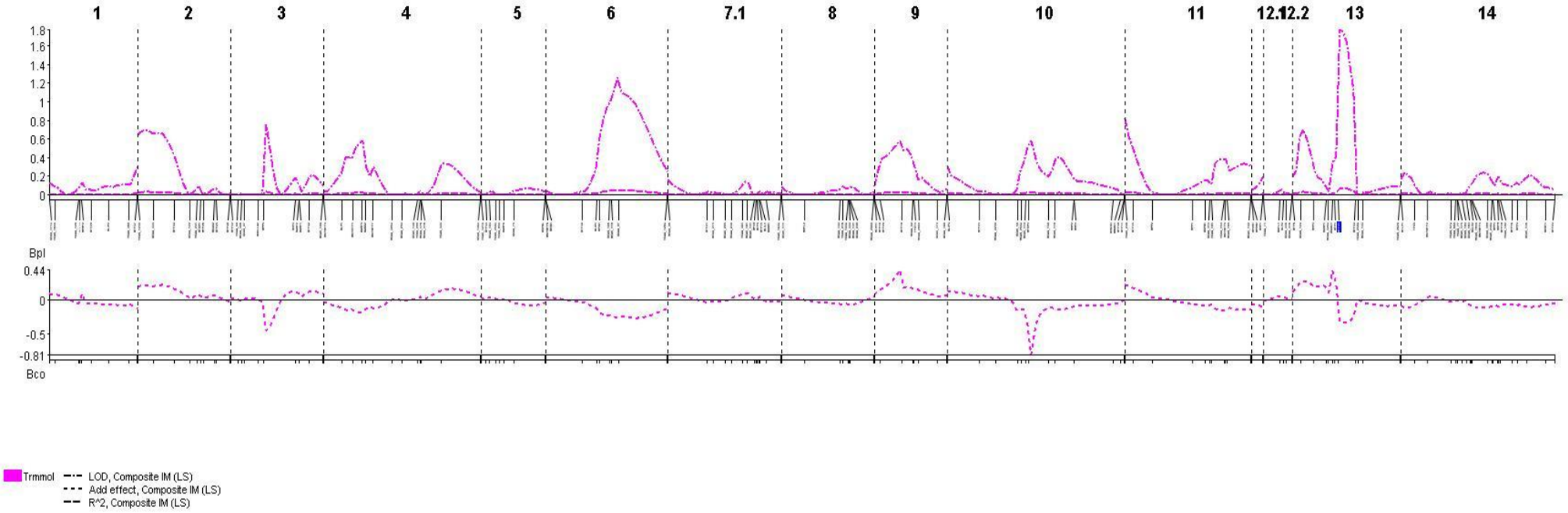

Figure 7.11: Leaf transpiration scan of a test statistic for sCIM. A total of 162 markers covering the whole genome were used as background markers. LOD support intervals were calculated from the CIM results. Genome-wide threshold LOD values to declare a QTL to be significant were determined with 500 permutations. The threshold values obtained with 500 permutations were alpha 0.10 = 2.693 alpha 0.05 = 3.199, alpha 0.01 = 4.443. Default measurements were used in QGENE to select cofactors. Selected background locus included BTF41 13 41.9.

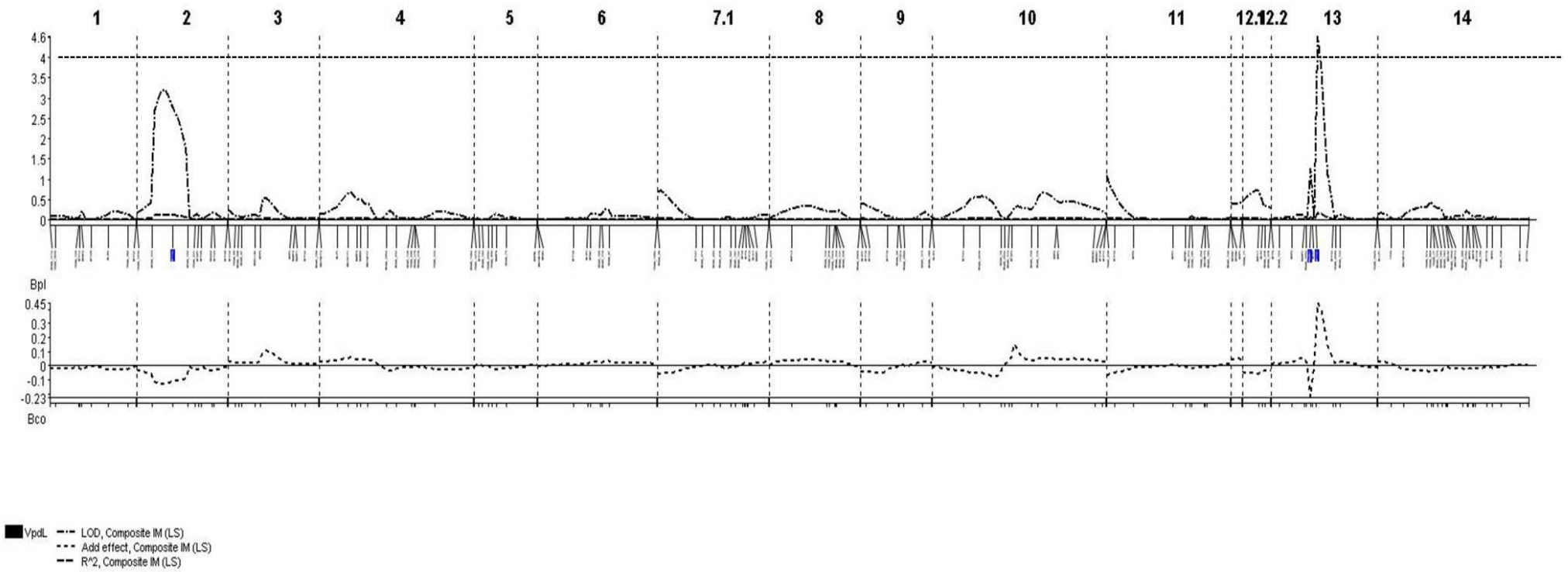

Figure 7.12: Vapour pressure deficit of the leaf test statistic for sCIM. A total of 162 markers covering the whole genome were used as background markers. LOD support intervals were calculated from the CIM results. Genome-wide threshold LOD values to declare a QTL to be significant were determined with 500 permutations. The threshold values obtained with 500 permutations were alpha 0.10 = 2.881, alpha 0.05 = 3.564 and alpha 0.01 = 5.739. Default measurements were used in QGENE to select cofactors. Selected background locus included BTF44 2 20.9, BNAM1 13 39.3, BTF41 13 41.9.

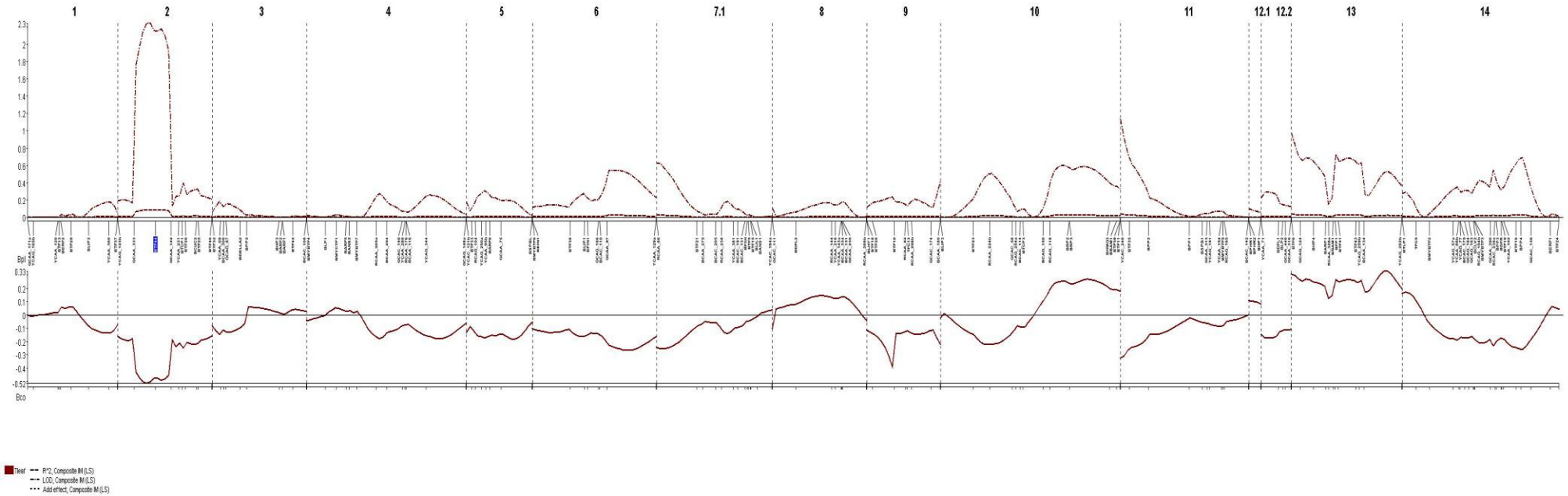

Figure 7.13: Temperature of the leaf test statistic for sCIM. A total of 162 markers covering the whole genome were used as background markers. LOD support intervals were calculated from the CIM results. Genome-wide threshold LOD values to declare a QTL to be significant were determined with 500 permutations. The threshold values obtained with 500 permutations were alpha 0.10 = 2.948, alpha 0.05 = 3.831, and alpha 0.01 = 7.117. Default measurements were used in QGENE to select cofactors. Selected background locus included BTF44 2 20.9.

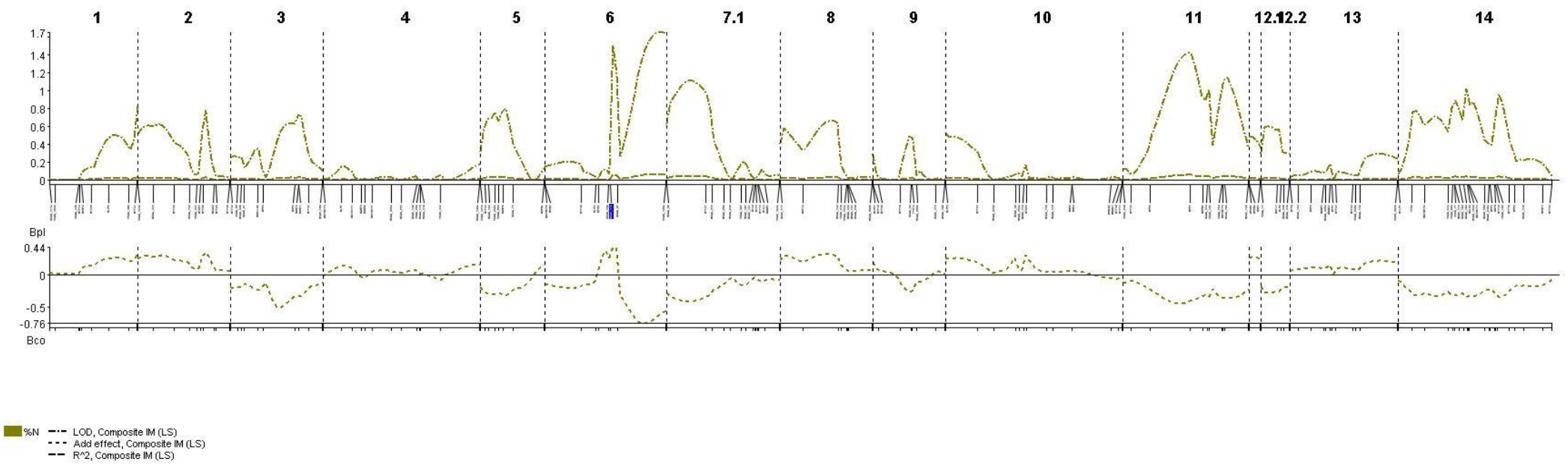

Figure 7.14: Total leaf nitrogen test statistic for sCIM. A total of 162 markers covering the whole genome were used as background markers. LOD support intervals were calculated from the CIM results. Genome-wide threshold LOD values to declare a QTL to be significant were determined with 500 permutations. The threshold values obtained with 500 permutations were alpha 0.10 = 2.759, alpha 0.05 = 3.165 and alpha 0.01 = 3.894. Default measurements were used in QGENE to select cofactors. Selected background locus included BCAC_110 6 37.6.

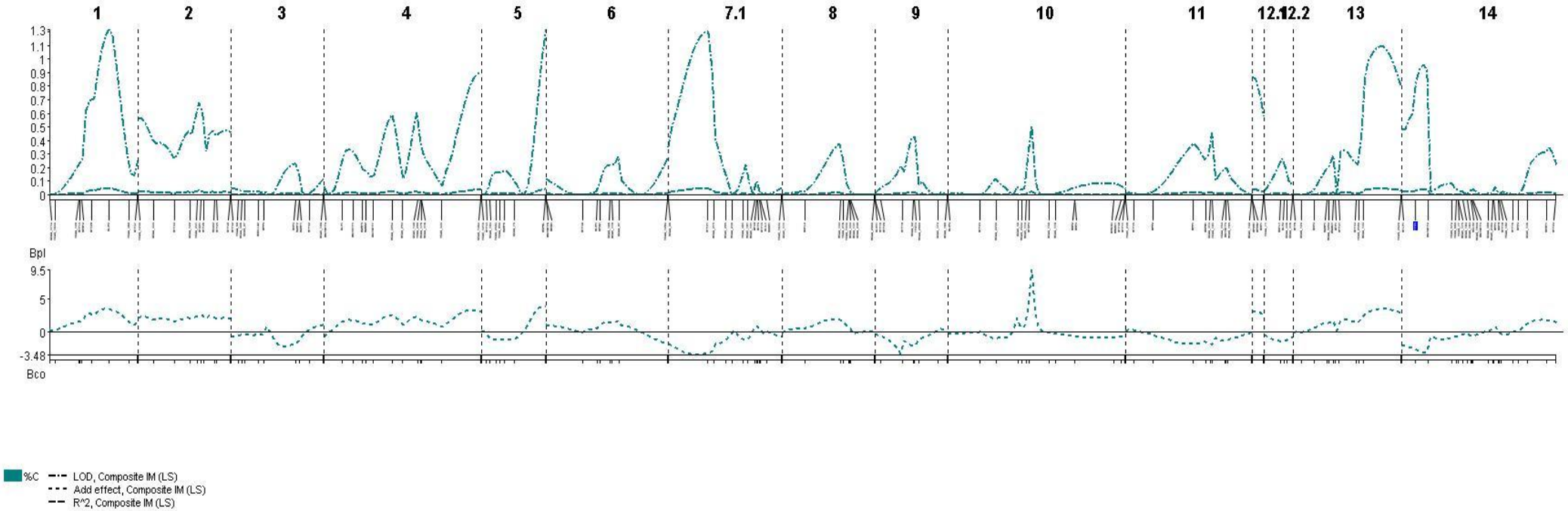

Figure 7.15: Total leaf carbon test statistic for sCIM. A total of 162 markers covering the whole genome were used as background markers. LOD support intervals were calculated from the CIM results. Genome-wide threshold LOD values to declare a QTL to be significant were determined with 500 permutations. The threshold values obtained with 500 permutations were alpha 0.10 = 2.925, alpha 0.05 = 3.223 and alpha 0.01 = 4.062. Default measurements were used in QGENE to select cofactors. Selected background locus included TFC5 14 7.8.

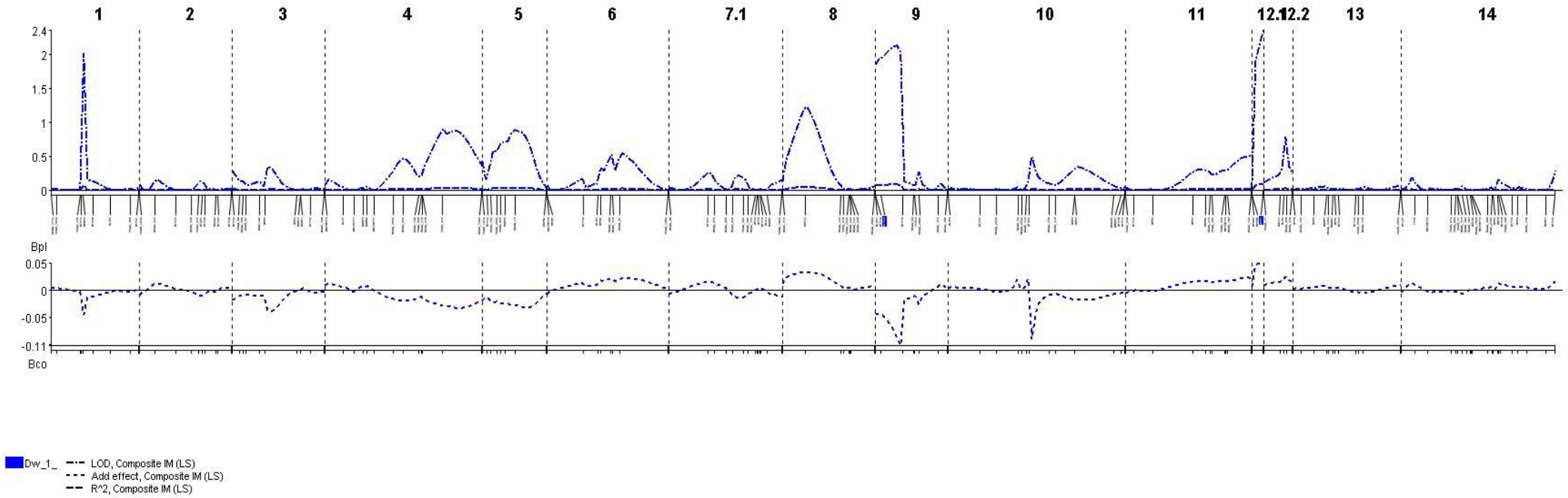

Figure 7.16: Total leaf dry weight test statistic for sCIM. A total of 162 markers covering the whole genome were used as background markers. LOD support intervals were calculated from the CIM results. Genome-wide threshold LOD values to declare a QTL to be significant were determined with 500 permutations. The threshold values obtained with 500 permutations were alpha 0.10 = 2.969, alpha 0.05 = 3.483 and alpha 0.01 = 4.407. Default measurements were used in QGENE to select cofactors. Selected background locus included BTF28 9 3.3, BHP1 12.1 6.9.

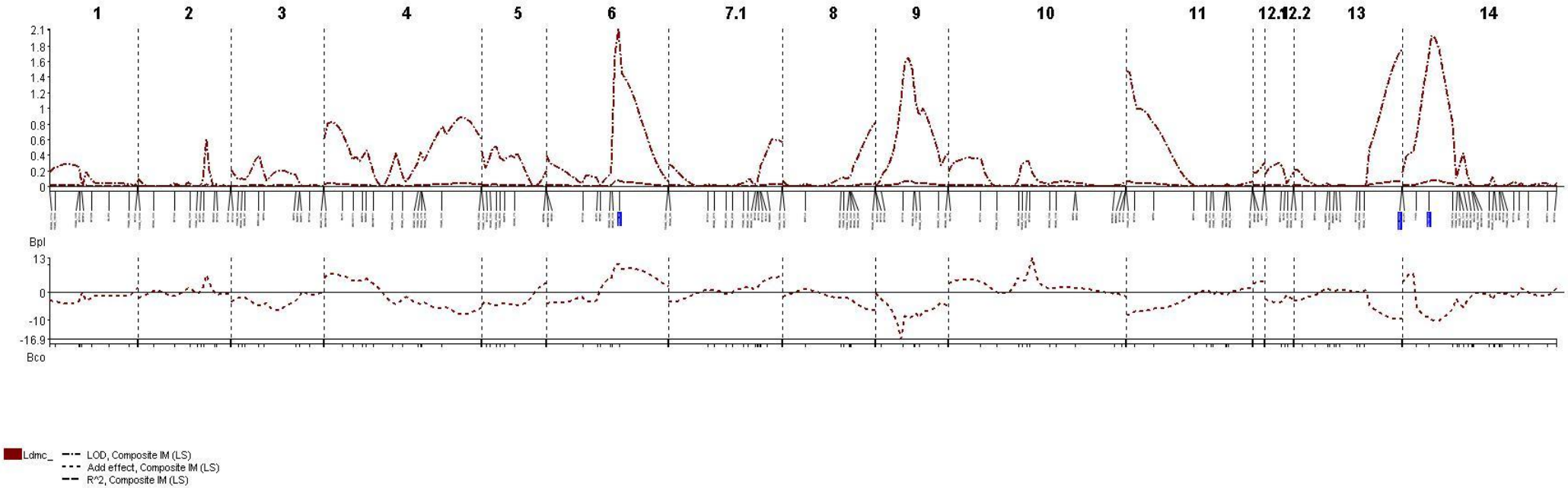

Figure 7.17: Leaf dry matter content test statistic for sCIM. A total of 162 markers covering the whole genome were used as background markers. LOD support intervals were calculated from the CIM results. Genome-wide threshold LOD values to declare a QTL to be significant were determined with 500 permutations. The threshold values obtained with 500 permutations were alpha 0.10 = 2.735, alpha 0.05 = 3.192 and alpha 0.01 = 4.525. Default measurements were used in QGENE to select cofactors. Selected background locus included GCAA_97 6 41.5, YCAG_282b 13 77.1, BMYBTF2 14 15.1.

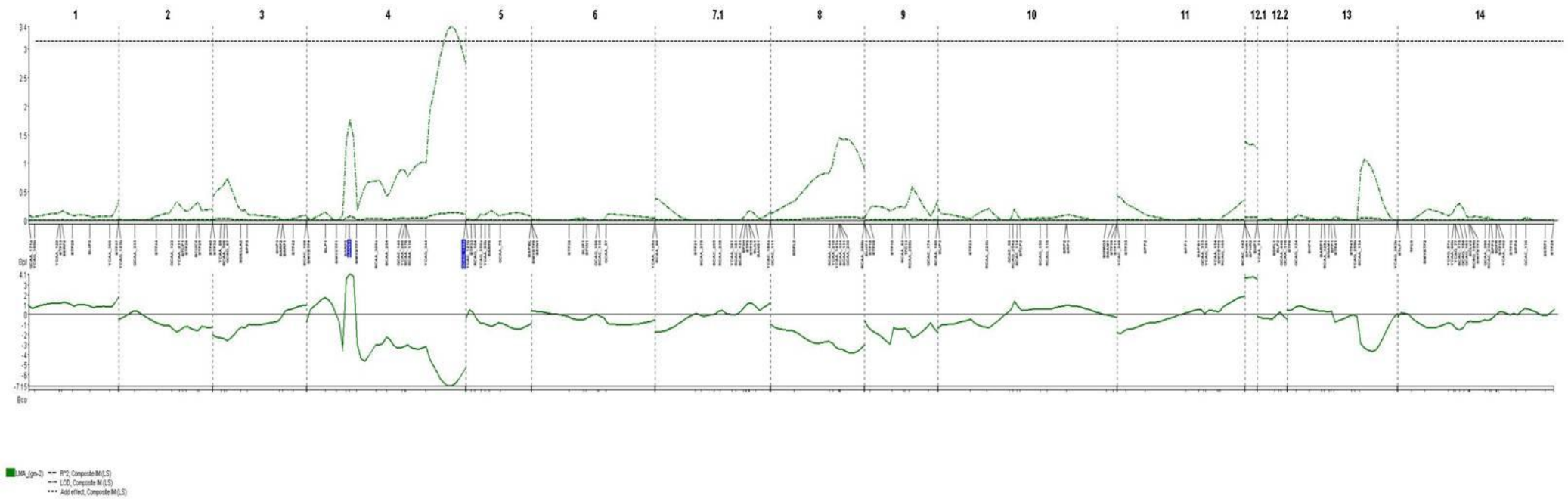

Figure 7.18: Leaf mass area test statistic for sCIM. A total of 162 markers covering the whole genome were used as background markers. LOD support intervals were calculated from the CIM results. Genome-wide threshold LOD values to declare a QTL to be significant were determined with 500 permutations. The threshold values obtained with 500 permutations were alpha 0.10 = 2.623, alpha 0.05 = 3.135 and alpha 0.01 = 4.05. Default measurements were used in QGENE to select cofactors. Selected background locus included BANS2 4 23.7, GCAG_148a 4 88.2.

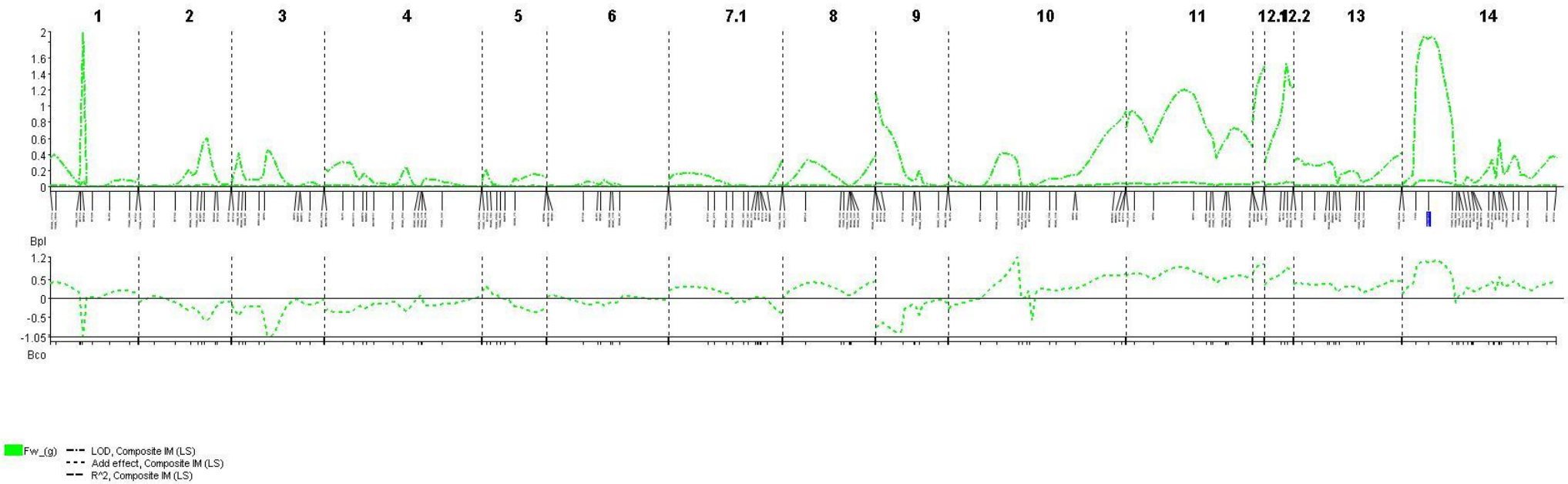

Figure 7.19: Fresh leaf weight test statistic for sCIM. A total of 162 markers covering the whole genome were used as background markers. LOD support intervals were calculated from the CIM results. Genome-wide threshold LOD values to declare a QTL to be significant were determined with 500 permutations. The threshold values obtained with 500 permutations were alpha 0.10 = 2.95, alpha 0.05 = 3.532 and alpha 0.01 = 4.968. Default measurements were used in QGENE to select cofactors. Selected background locus included BMYBTF2 14 15.1.

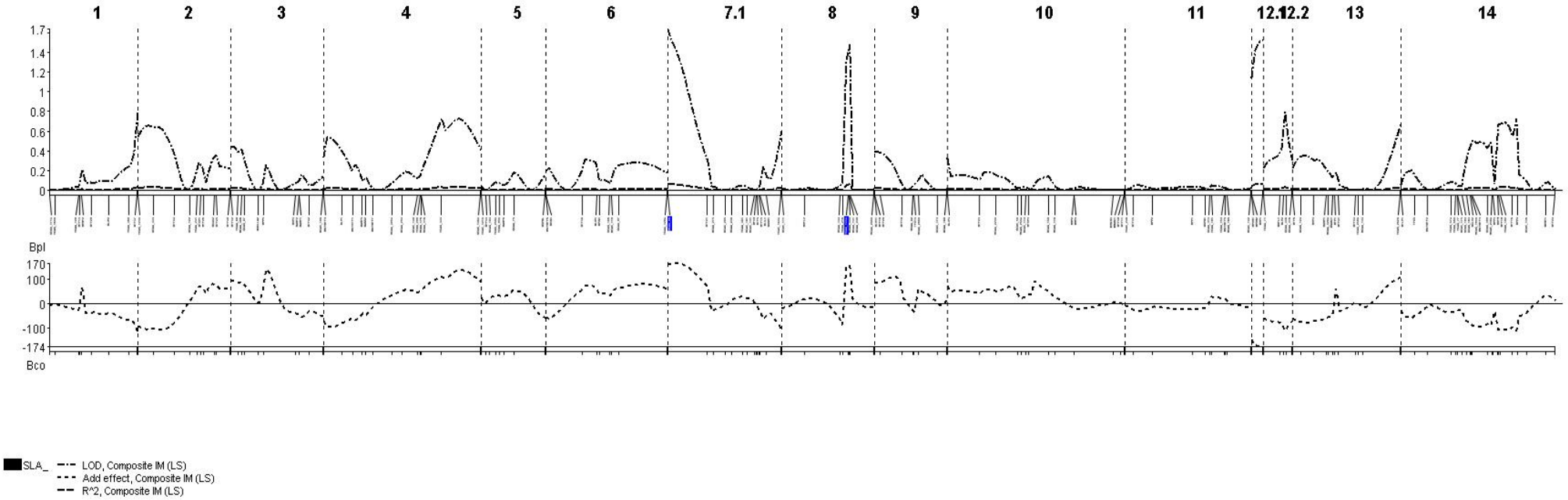

Figure 7.20: Specific leaf area test statistic for sCIM. A total of 162 markers covering the whole genome were used as background markers. LOD support intervals were calculated from the CIM results. Genome-wide threshold LOD values to declare a QTL to be significant were determined with 500 permutations. The threshold values obtained with 500 permutations were alpha 0.10 = 3.094, alpha 0.05 = 3.915 and alpha 0.01 = 6.852. Default measurements were used in QGENE to select cofactors. Selected background locus included RCAA_86 7.1 0.0, YCAA_143a 8 56.9.

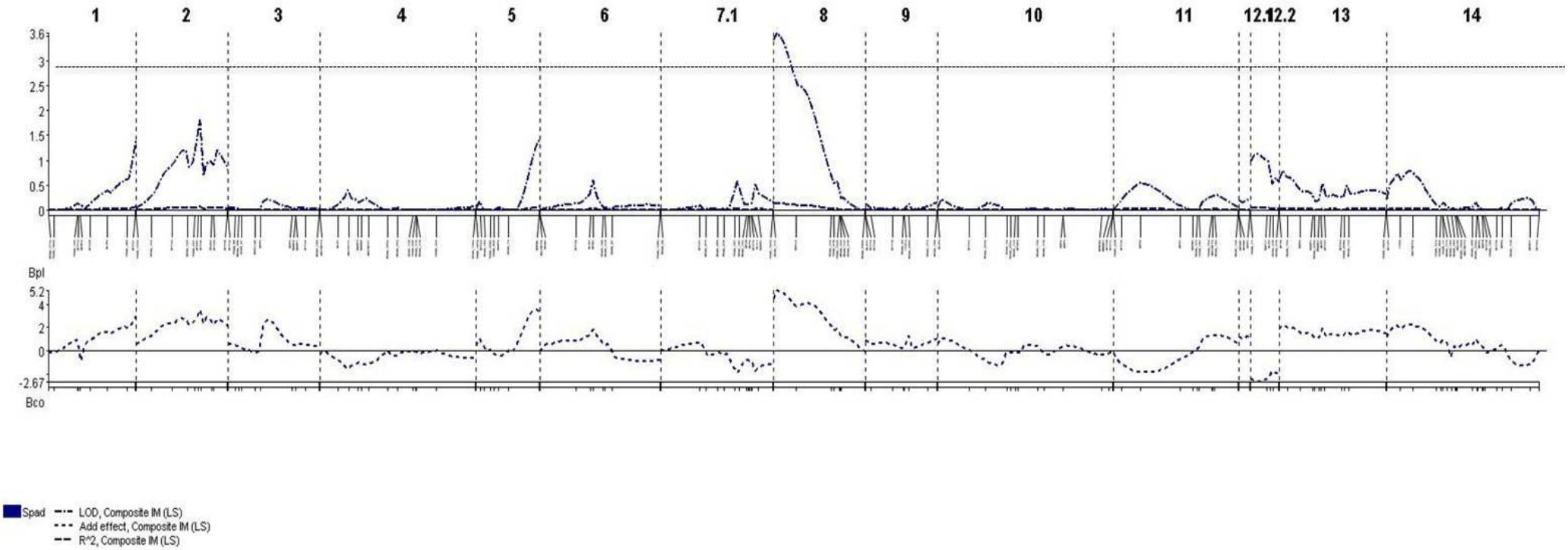

Figure 7.21: Spad content scan test statistic for sCIM. A total of 162 markers covering the whole genome were used as background markers. LOD support intervals were calculated from the CIM results. Genome-wide threshold LOD values to declare a QTL to be significant were determined with 500 permutations. The threshold values obtained with 500 permutations were alpha 0.10 = 2.447, alpha 0.05 = 2.682 and alpha 0.01 = 3.244. Default measurements were used in QGENE to select cofactors. Selected background locus included BTF41 13 41.9.

7.3.3. Leaf size and shape traits:

Estimation of QTL positions by simplified composite interval mapping for leaf shape and area attribute traits:

A very prominent peak was observed for leaf area on chromosome 9 at 0 – 34 cM (Figure 7.22). This QTL explained 12 % of the variation seen for this trait.

The current analysis revealed significant QTLs for traits related to leaf shape including vertical symmetry, indent width mean and indent depth mean. A single QTL for leaf vertical symmetry was located on chromosome 10 at 0 cM – 34 cM and explained 16.9 % of the total variation for this trait. The locus had a positive additive effect (Figure 7.27). Open reading frames for two of the SNP markers BL1P3 and BTF23 matched transcription factors in the *Arabidopsis* database, though not ones with an obvious link to margin growth regulation.

Another leaf shape QTL for indent width mean was found on chromosome 2 between 34 to 36cM explaining 14.3% of the total indent widths mean variation. The locus had negative additive effect (Figure 7.32). Chromosome 2 also harbored QTLs for depth of adaxial hypodermis, width of adaxial cell and abaxial trichome type. The clustering of the QTLs for indent width mean is consistent with the significant phenotypic correlations observed between indent width mean with width of adaxial cell and width of adaxial hypodermis traits in the mapping population ($p > 0.05$). The BTCP1 SNP linked to the QTL for Indent width mean BTCP1 is a TCP2 factor whose *Arabidopsis* ortholog is involved in cell differentiation, leaf development and morphogenesis.

QTL for indent depth mean was found on chromosome 8 between 54.7 – 56.7cM and explained 14.3 % of total indent depth mean variation. The locus had positive additive effect

(Figure 7.31). The BDFL2 SNP linked to the QTL for Indent depth mean had an ortholog in the *Arabidopsis* database that encodes an IAA-amido synthase involved in growth.

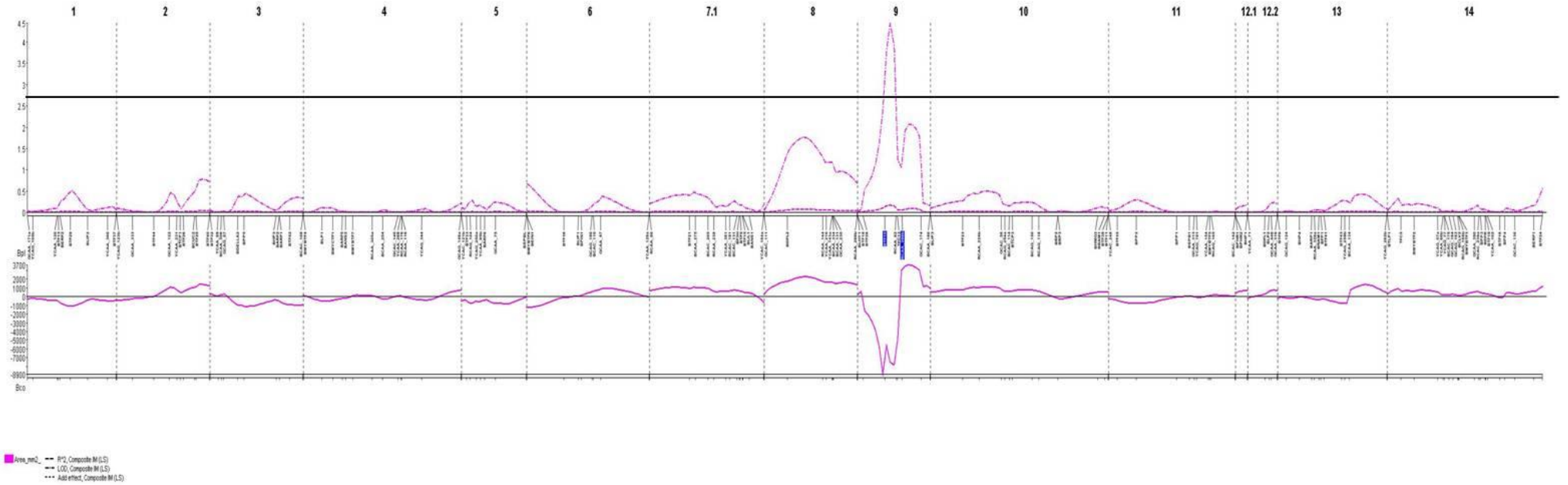

Figure 7.22: Area of leaf scan test statistic for sCIM. A total of 162 markers covering the whole genome were used as background markers. LOD support intervals were calculated from the CIM results. Genome-wide threshold LOD values to declare a QTL to be significant were determined with 500 permutations. The threshold values obtained with 500 permutations were alpha 0.10 = 2.447, alpha 0.05 = 2.682 and alpha 0.01 = 3.244. Default measurements were used in QGENE to select cofactors. Selected background locus included BTF41 13 41.9.

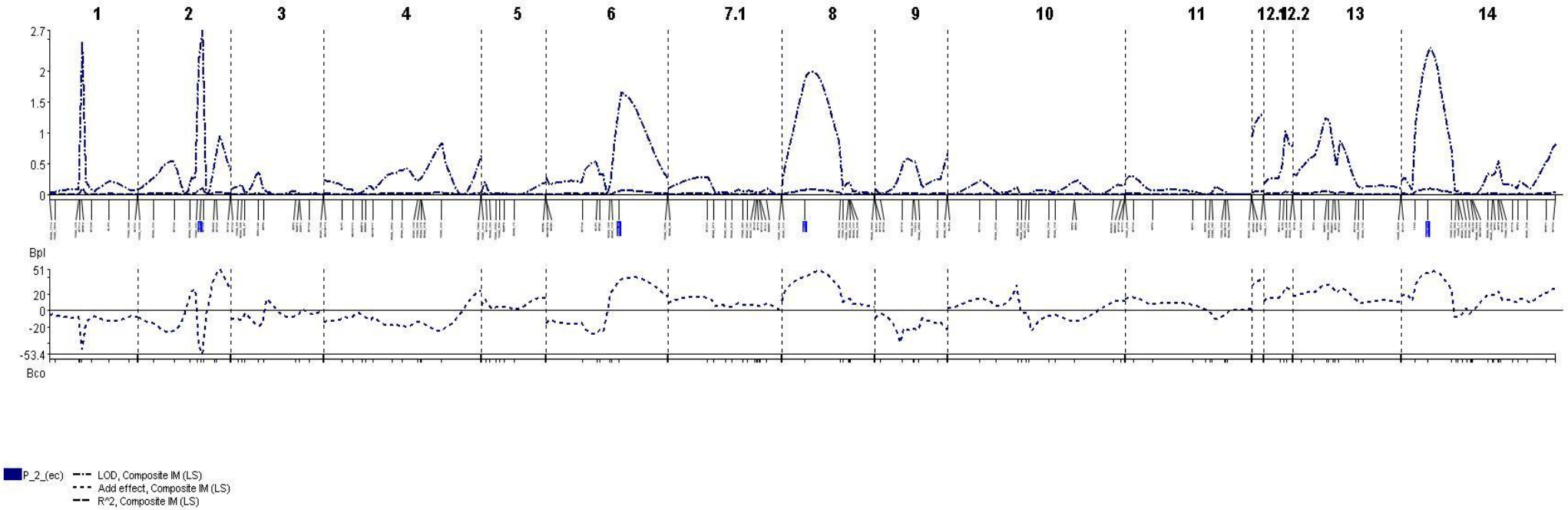

Figure 7.23: Perimeter scan test statistic for sCIM. A total of 162 markers covering the whole genome were used as background markers. LOD support intervals were calculated from the CIM results. Genome-wide threshold LOD values to declare a QTL to be significant were determined with 500 permutations. The threshold values obtained with 500 permutations were alpha 0.10 = 2.785, alpha 0.05 = 3.221 and alpha 0.01 = 3.992. Default measurements were used in QGENE to select cofactors. Selected background locus included BTCP1 2 35.6, GCAA_97 6 41.5, BDFL2 8 31.9, BMYBTF2 14 15.1.

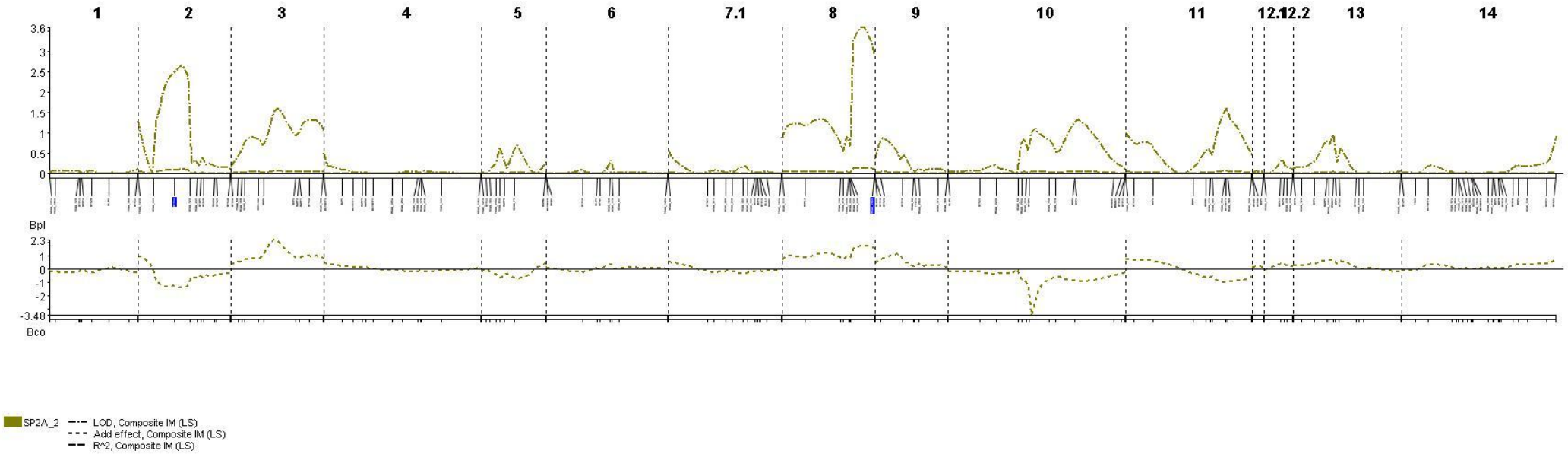

Figure 7.24: Perimeter area ratio scan test statistic for sCIM. A total of 162 markers covering the whole genome were used as background markers. LOD support intervals were calculated from the CIM results. Genome-wide threshold LOD values to declare a QTL to be significant were determined with 500 permutations. The threshold values obtained with 500 permutations were alpha 0.10 = 2.758, alpha and 0.05 = 3.364 and alpha 0.01 = 4.602. Default measurements were used in QGENE to select cofactors. Selected background locus included BTF44 2 20.9, RCAA_288b 8 70.9.

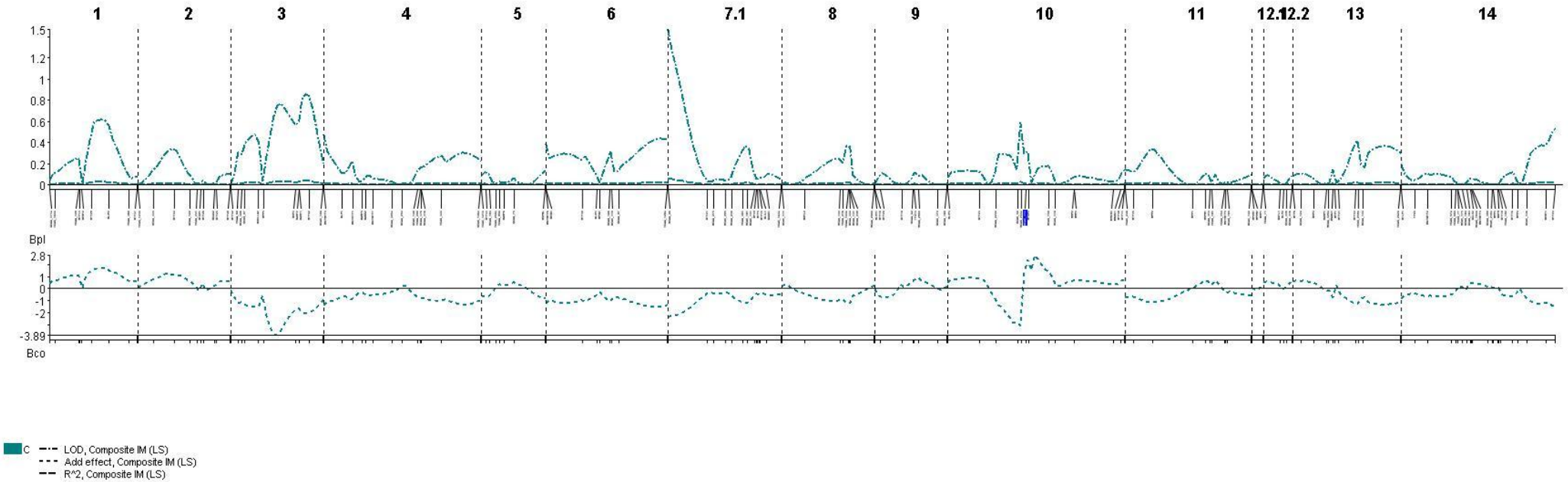

Figure 7.25: Circularity scan test statistic for sCIM. A total of 162 markers covering the whole genome were used as background markers. LOD support intervals were calculated from the CIM results. Genome-wide threshold LOD values to declare a QTL to be significant were determined with 500 permutations. The threshold values obtained with 500 permutations were alpha 0.10 = 2.739, alpha 0.05 = 3.132 and alpha 0.01 = 4.183. Default measurements were used in QGENE to select cofactors. Selected background locus included BCAC_114 10 44.2.

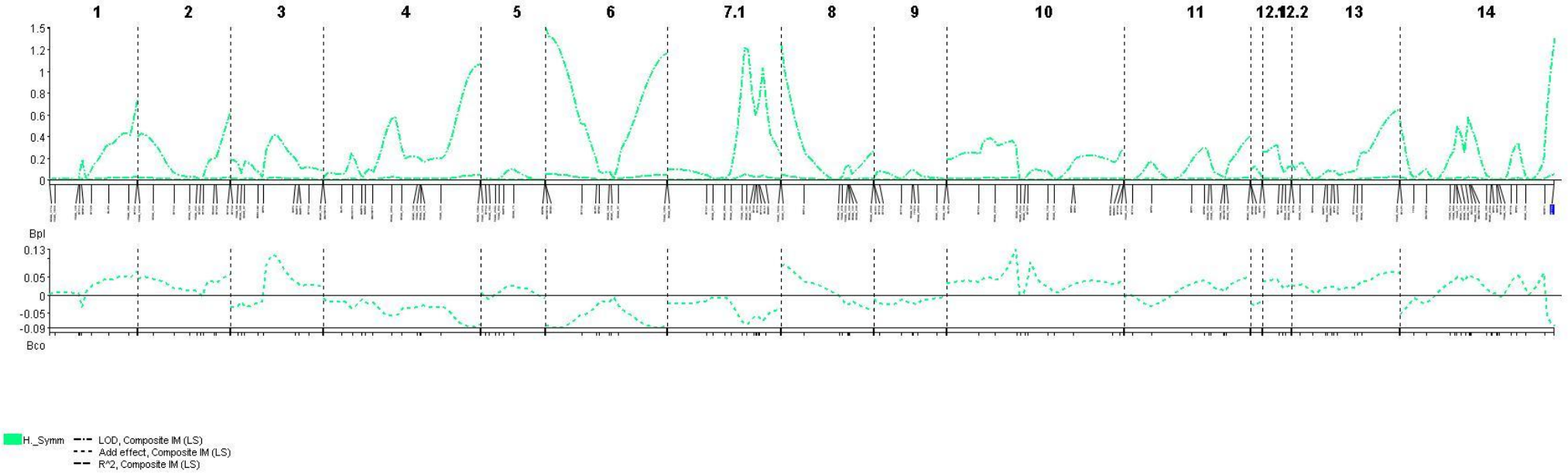

Figure 7.26: Horizontal symmetry scan test statistic for sCIM. A total of 162 markers covering the whole genome were used as background markers. LOD support intervals were calculated from the CIM results. Genome-wide threshold LOD values to declare a QTL to be significant were determined with 500 permutations. The threshold values obtained with 500 permutations were alpha 0.10 = 2.854, alpha 0.05 = 3.256 and alpha 0.01 = 3.788. Default measurements were used in QGENE to select cofactors. Selected background locus included BTF24 14 86.6.

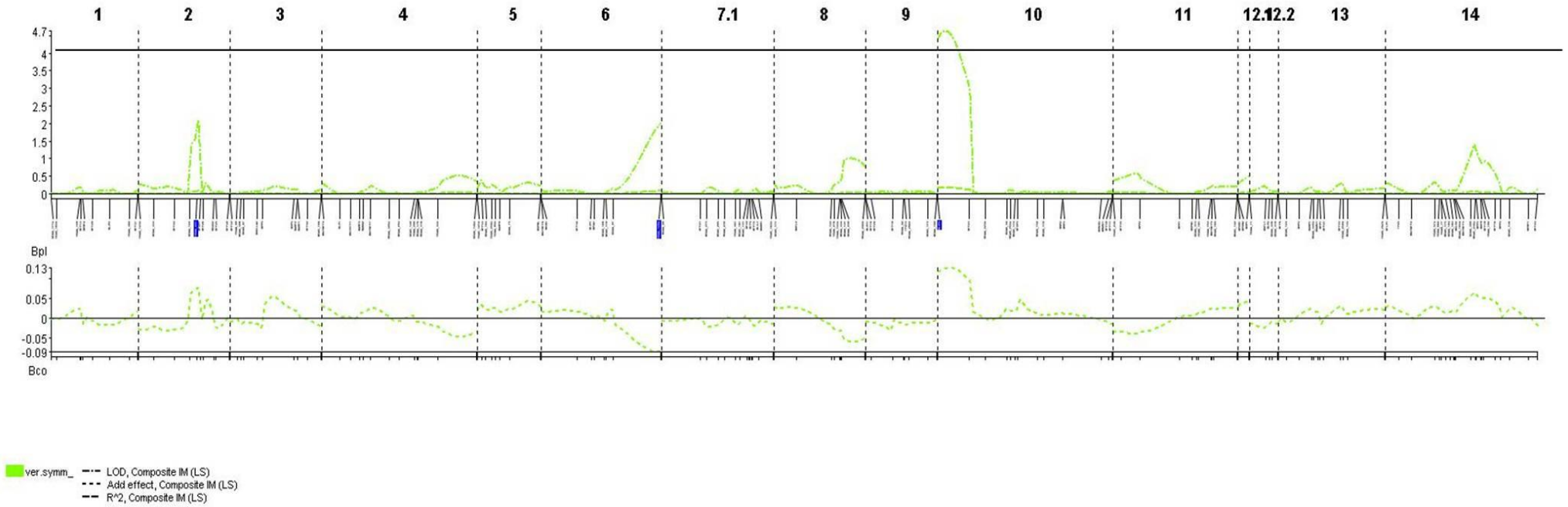

Figure 7.27: Vertical symmetry scan test statistic for sCIM. A total of 162 markers covering the whole genome were used as background markers. LOD support intervals were calculated from the CIM results. Genome-wide threshold LOD values to declare a QTL to be significant were determined with 500 permutations. The threshold values obtained with 500 permutations were alpha 0.10 = 2.654, alpha 0.05 = 3.12and alpha 0.01 = 4.06. Default measurements were used in QGENE to select cofactors. Selected background locus included BCAA_254 4 44.5.

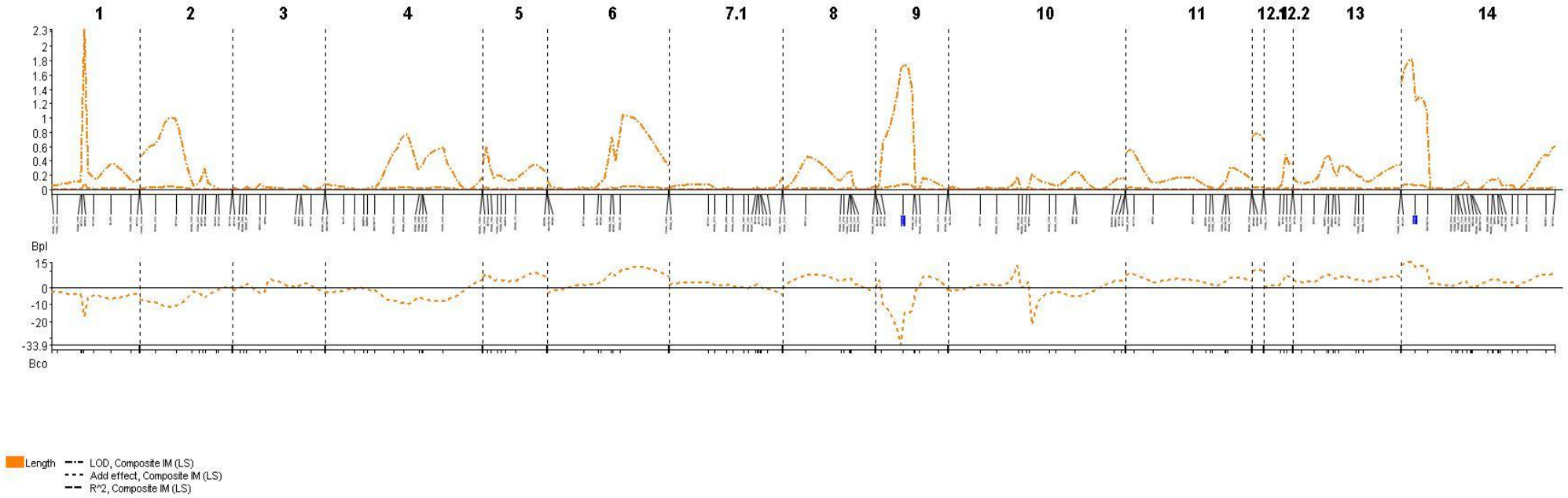

Figure 7.28: Leaf lengths scan test statistic for sCIM. A total of 162 markers covering the whole genome were used as background markers. LOD support intervals were calculated from the CIM results. Genome-wide threshold LOD values to declare a QTL to be significant were determined with 500 permutations. The threshold values obtained with 500 permutations were alpha 0.10 = 2.661, alpha 0.05 = 3.282 and alpha 0.01 = 4.091. Default measurements were used in QGENE to select cofactors. Selected background locus included BTF10 9 15.5, TFC5 14 7.8.

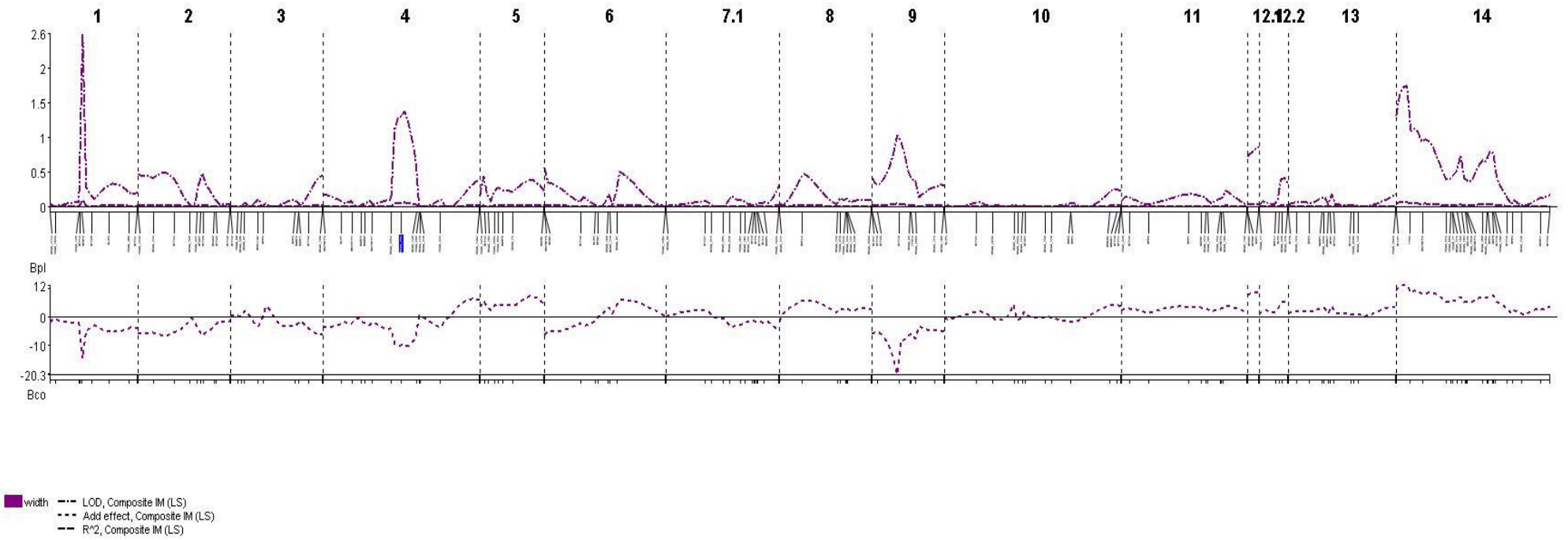

Figure 7.29: Leaf width scan test statistic for sCIM. A total of 162 markers covering the whole genome were used as background markers. LOD support intervals were calculated from the CIM results. Genome-wide threshold LOD values to declare a QTL to be significant were determined with 500 permutations. The threshold values obtained with 500 permutations were alpha 0.10 = 2.654, alpha 0.05 = 3.12 and alpha 0.01 = 4.064. Default measurements were used in QGENE to select cofactors. Selected background locus included BCAA_254 4 44.5.

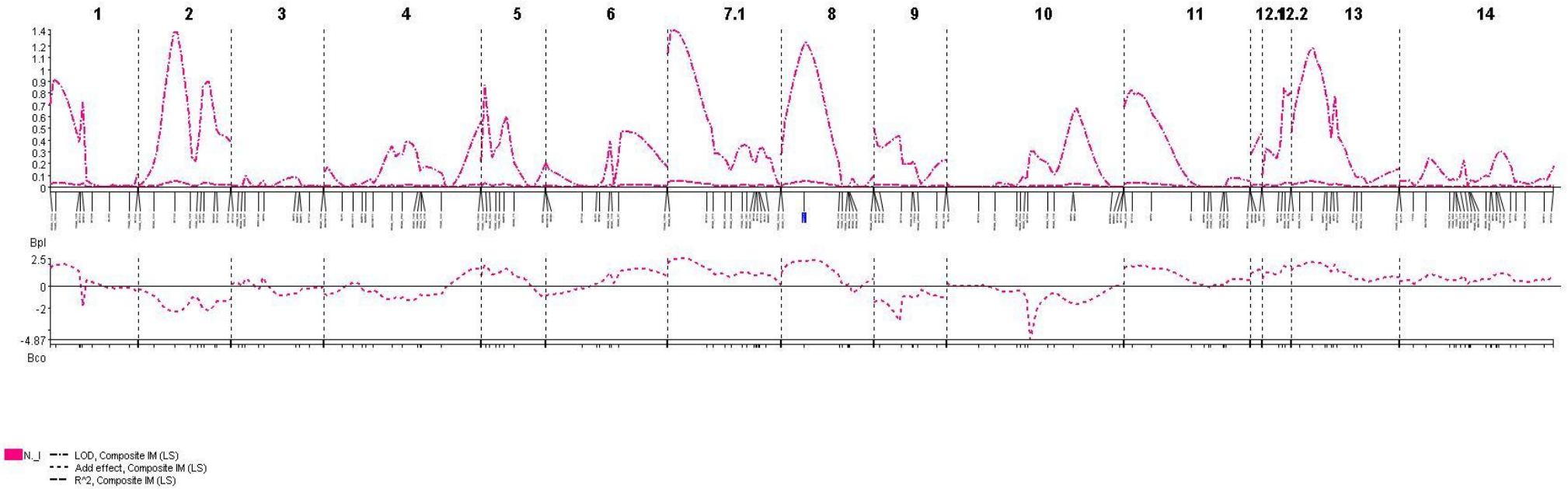

Figure 7.30: Number of indents scan test statistic for sCIM. A total of 162 markers covering the whole genome were used as background markers. LOD support intervals were calculated from the CIM results. Genome-wide threshold LOD values to declare a QTL to be significant were determined with 500 permutations. The threshold values obtained with 500 permutations were alpha 0.10 = 2.697, alpha 0.05 = 3.135 and alpha 0.01 = 4.498. Default measurements were used in QGENE to select cofactors. Selected background locus included BDFL2 8 31.9.

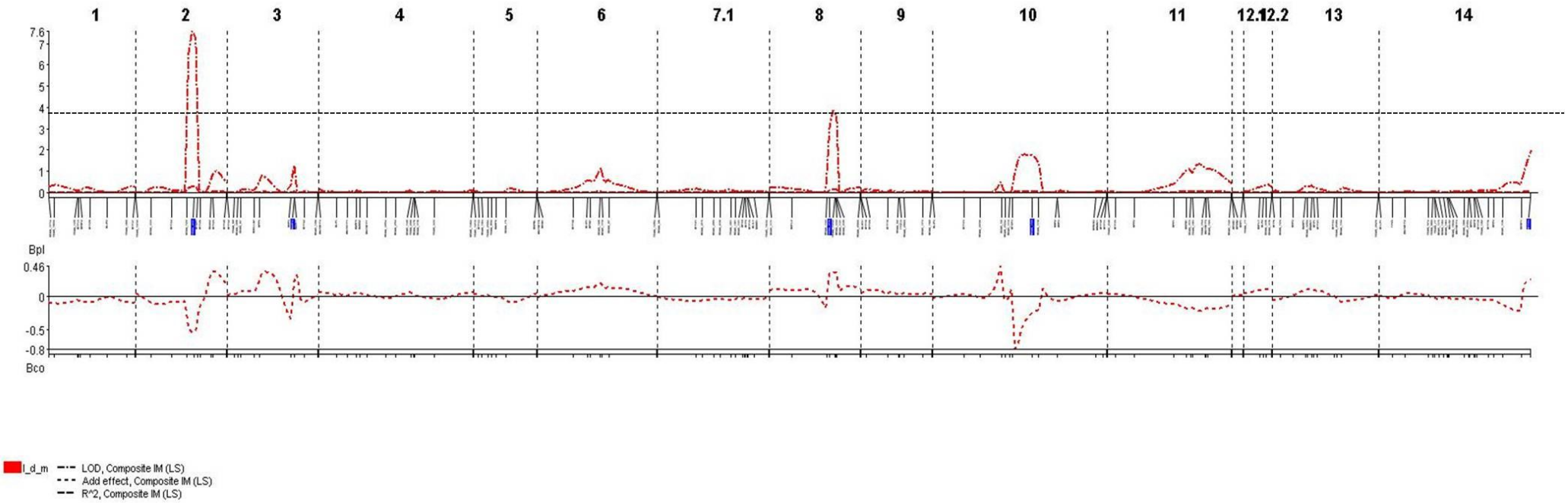

Figure 7.31: Indent depth mean scan test statistic for sCIM. A total of 162 markers covering the whole genome were used as background markers. LOD support intervals were calculated from the CIM results. Genome-wide threshold LOD values to declare a QTL to be significant were determined with 500 permutations. The threshold values obtained with 500 permutations were alpha 0.10 = 2.861, alpha 0.05 = 3.237 and alpha 0.01 = 4.646. Default measurements were used in QGENE to select cofactors. Selected background locus included YCAA_231 2 33.9, BARF2 3 63.3, YCAA_216 8 53.6, BCAG_150 10 56.9, BTF24 14 86.6.

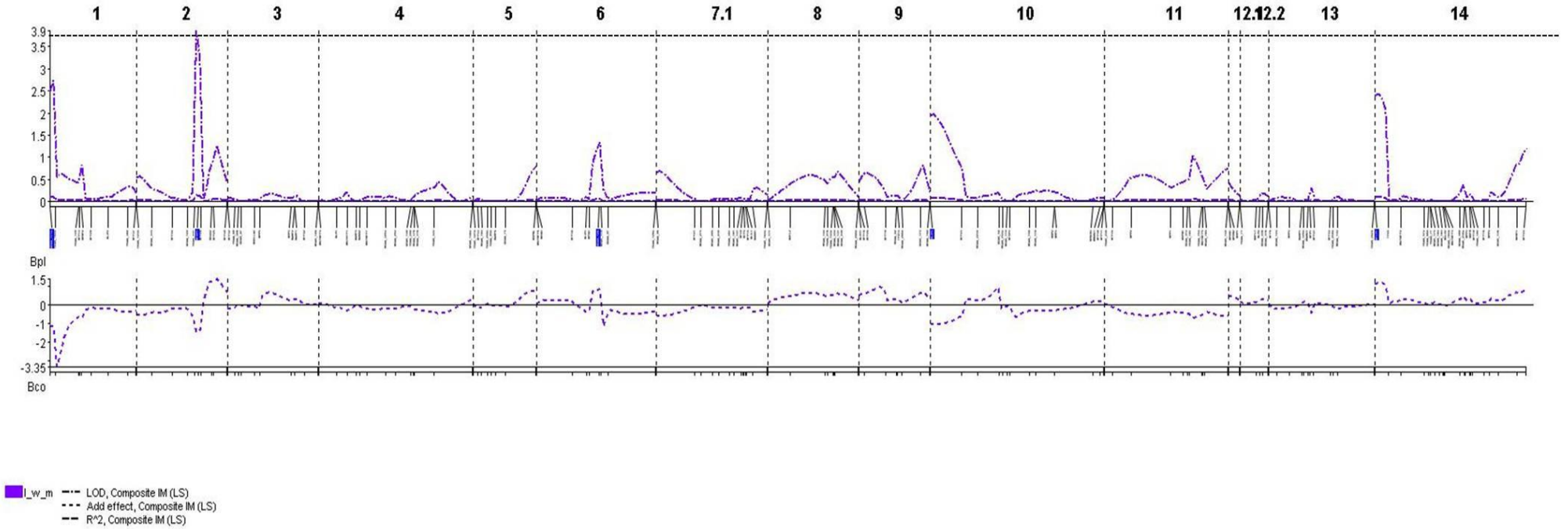

Figure 7.32: Indent width mean scan test statistic for sCIM. A total of 162 markers covering the whole genome were used as background markers. LOD support intervals were calculated from the CIM results. Genome-wide threshold LOD values to declare a QTL to be significant were determined with 500 permutations. The threshold values obtained with 500 permutations were alpha 0.10 = 2.826, alpha 0.05 = 3.178 and alpha 0.01 = 3.877. Default measurements were used in QGENE to select cofactors. Selected background locus included GCAA_171a 1 1.2, BTCP1 2 35.6, GCAG_186 6 36.6, BLIP3 10 0.0, BTLP1 14 0.0.

7.3.4. Micromorphological traits:

Estimation of QTL positions by chi- squared tests for independence:

The chi-squared tests for independence of trait scores tabulated against the genotypes at each marker in turn resolved a QTL for abaxial trichomes. A single marker RCAA_288b present on chromosome 8 at a position of 70.9 cM was found to be significantly associated with the presence of trichomes on the abaxial leaf surface.

Estimation of QTL positions by simplified composite interval mapping:

Significant QTLs were found for six of the continuous traits measured in this category. A single QTL for depth of adaxial hypodermis was located on chromosome 8 at an interval of 66.7 - 70.7 cM and explained 13.3% of the total variation for this trait (Figure 7.37). The locus had a negative effect. QTLs were also resolved for width of adaxial and abaxial cells. A QTL for width of adaxial cell was found on chromosome 8 at 70.7cM explaining 12.6 % of total variation. The locus had negative additive effect (Figure 7.40). Another QTL for width of abaxial cell was found on chromosome 2 at an interval of 20 – 22cM and explained 11% of the total variation. The locus had negative additive effect. The SNP marker BTF44, linked to this QTL is an ortholog of a TCP family transcription factor possibly involved in growth regulation in *Arabidopsis* (Figure 7.41).

The analysis also identified QTLs for stomatal patterning and vascularisation traits on chromosome number 13. At position of 42cM a QTL for number of stomatal clusters per mm^2 of the leaf which explained a total of 19 % of variation was identified (Figure 7.45). The linked SNP marker BTF41 encodes a family of actin bundlers, predominantly expressed in *Arabidopsis* pollen and is unlikely to be directly involved in this trait. Closely linked, at 38

cM on chromosome 13 a QTL for the number of central vascular bundles was found which explained 14% of the total variation. The locus had negative additive effect (Figure 7.60). A QTL for the width of inner vascular bundle was found at a position of 8- 18cM on the same chromosome and explained 14% of the variation. The locus had negative additive effect (Figure 7.63).

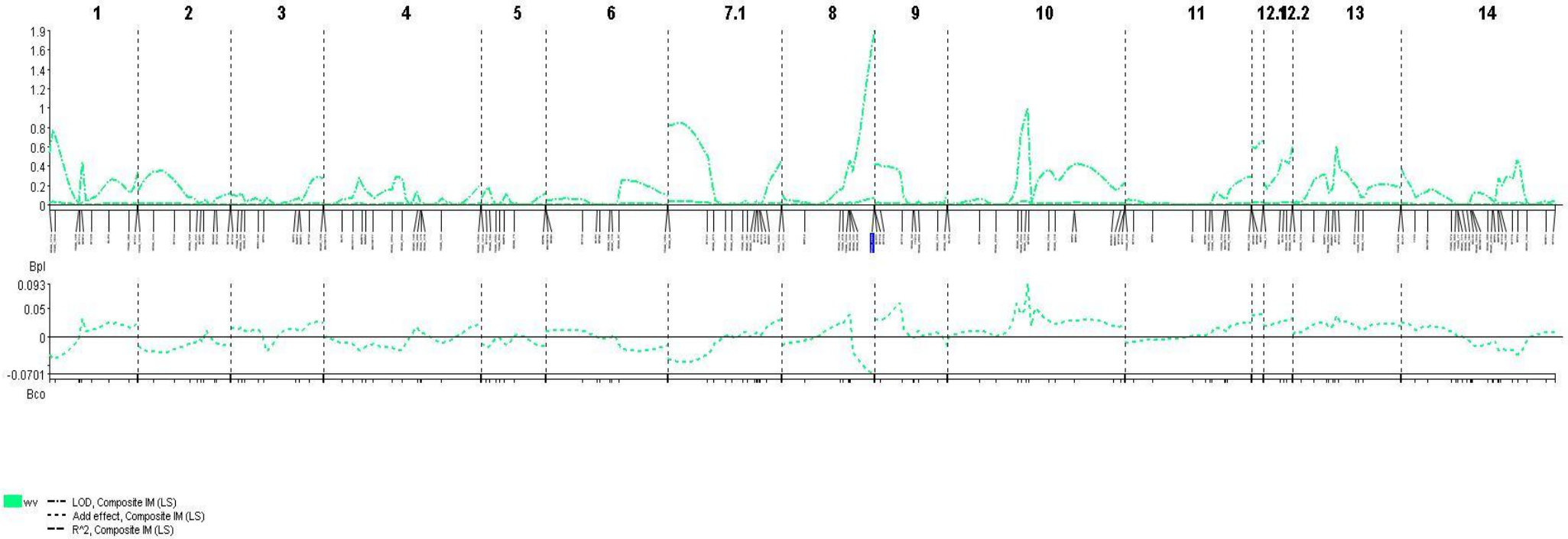

Figure 7.33: Width at vein scan of a test statistic scan of a test statistic for sCIM. A **total of 162 markers covering the whole genome were used as background markers. LOD support intervals were calculated from the CIM results. Genome-wide threshold LOD values to declare a QTL to be significant were determined with 500 permutations. The threshold values obtained with 500 permutations were alpha 0.10 = 2.693, alpha 0.05 = 3.13 and alpha 0.01 = 4.101. Default measurements were used in QGENE to select cofactors. Selected background locus included RCAA_288b 8 70.9.**

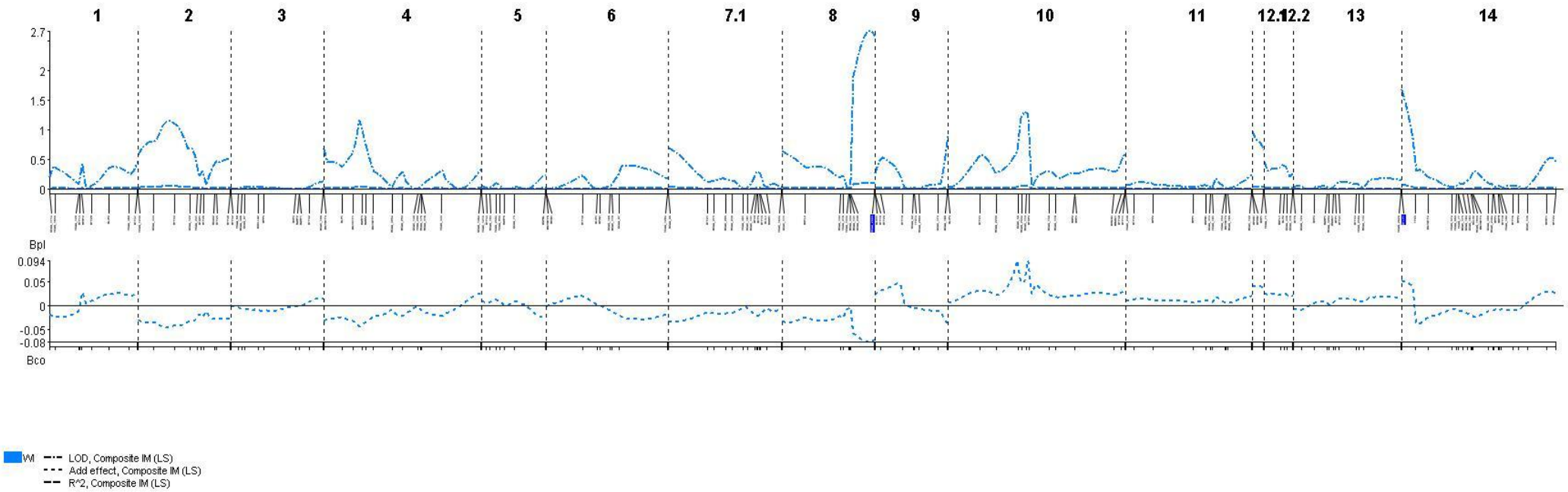

Figure 7.34: Width at lamina scan of a test statistic scan of a test statistic for sCIM. A total of 162 markers covering the whole genome were used as background markers. LOD support intervals were calculated from the CIM results. Genome-wide threshold LOD values to declare a QTL to be significant were determined with 500 permutations. The threshold values obtained with 500 permutations were alpha 0.10 = 2.831, alpha 0.05 = 3.475 and alpha 0.01 = 4.743. Default measurements were used in QGENE to select cofactors. Selected background locus included RCAA_288b 8 70.9, BTLP1 14 0.0.

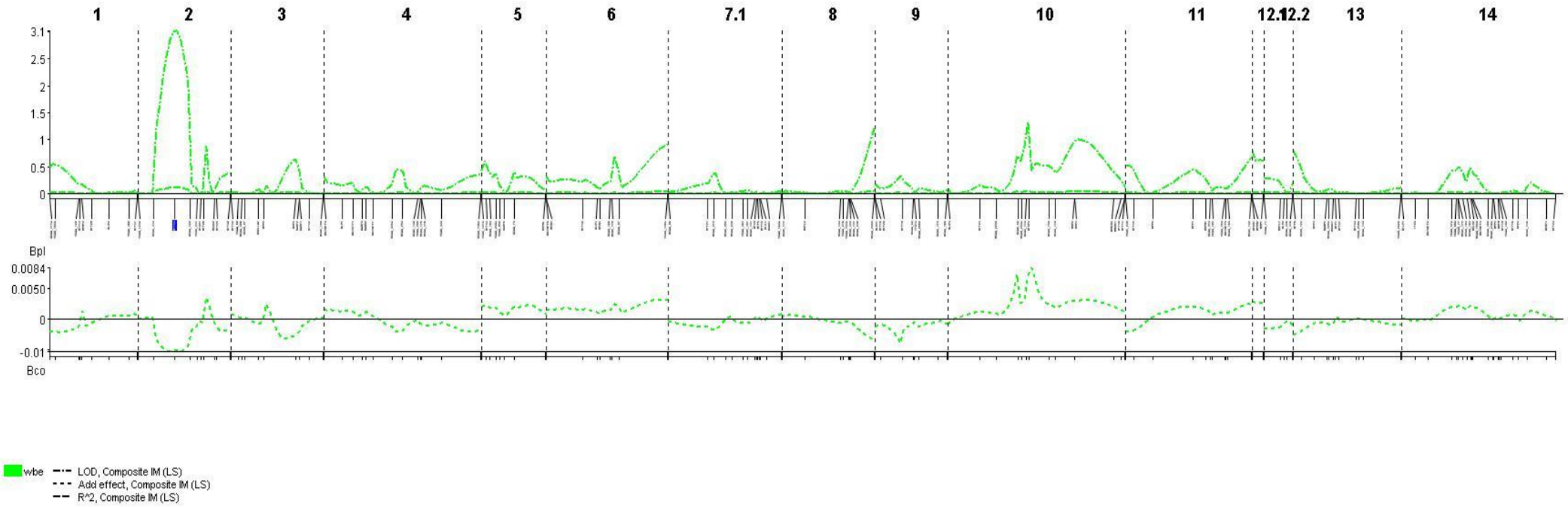

Figure 7.35: Width at adaxial epidermis scan of a test statistic for sCIM. A total of 162 markers covering the whole genome were used as background markers. LOD support intervals were calculated from the CIM results. Genome-wide threshold LOD values to declare a QTL to be significant were determined with 500 permutations. The threshold values obtained with 500 permutations were alpha 0.10 = 2.725, alpha 0.05 = 3.254 and alpha 0.01 = 4.172. Default measurements were used in QGENE to select cofactors. Selected background locus included RCAA_288b 8 70.9.

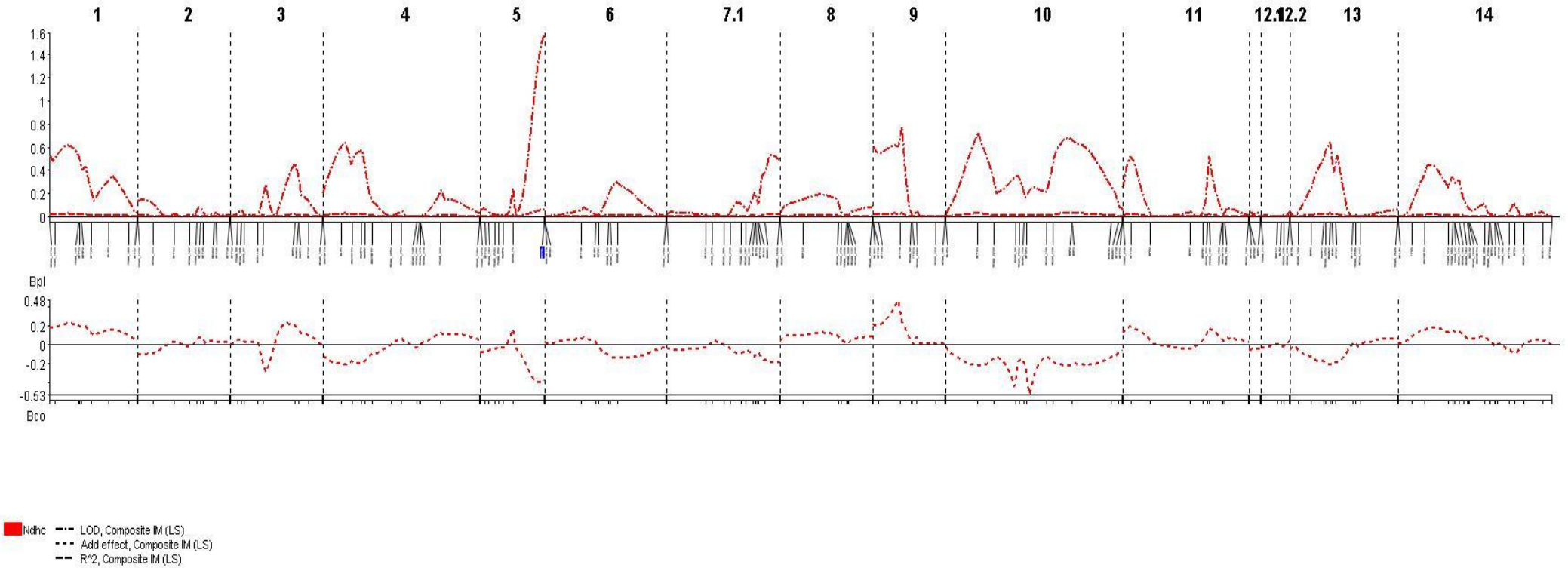

Figure 7.36: Number of adaxial hypoderm cells scan of a test statistic scan of a test statistic for sCIM. A total of 162 markers covering the whole genome were used as background markers. LOD support intervals were calculated from the CIM results. Genome-wide threshold LOD values to declare a QTL to be significant were determined with 500 permutations. The threshold values obtained with 500 permutations were alpha 0.10 = 2.666, alpha 0.05 = 3.084 ND alpha 0.01 = 4.106. Default measurements were used in QGENE to select cofactors. Selected background locus included BSPBL 5 36.4.

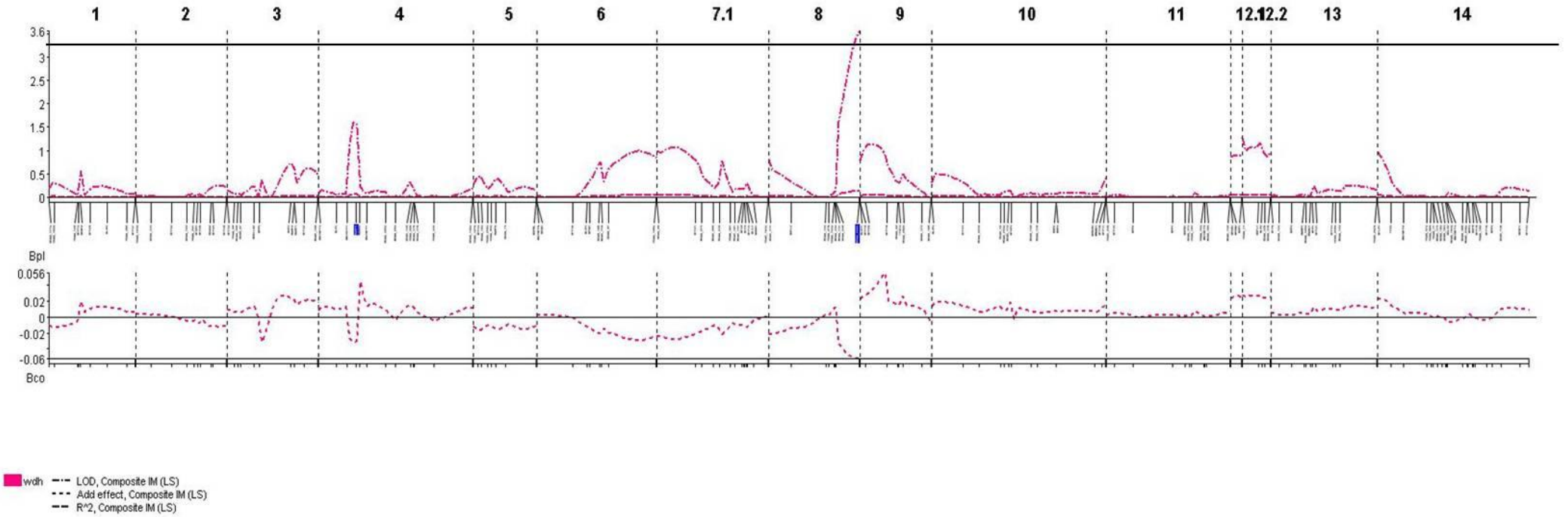

Figure 7.37: Depth of adaxial hypoderm scan of a test statistic scan of a test statistic for sCIM. A total of 162 markers covering the whole genome were used as background markers. LOD support intervals were calculated from the CIM results. Genome-wide threshold LOD values to declare a QTL to be significant were determined with 500 permutations. The threshold values obtained with 500 permutations were alpha 0.10 = 2.72, alpha 0.05 = 3.136 and alpha 0.01 = 3.8616. Default measurements were used in QGENE to select cofactors. Selected background locus included BARF5 4 22.0, RCAA_288b 8 70.9.

.

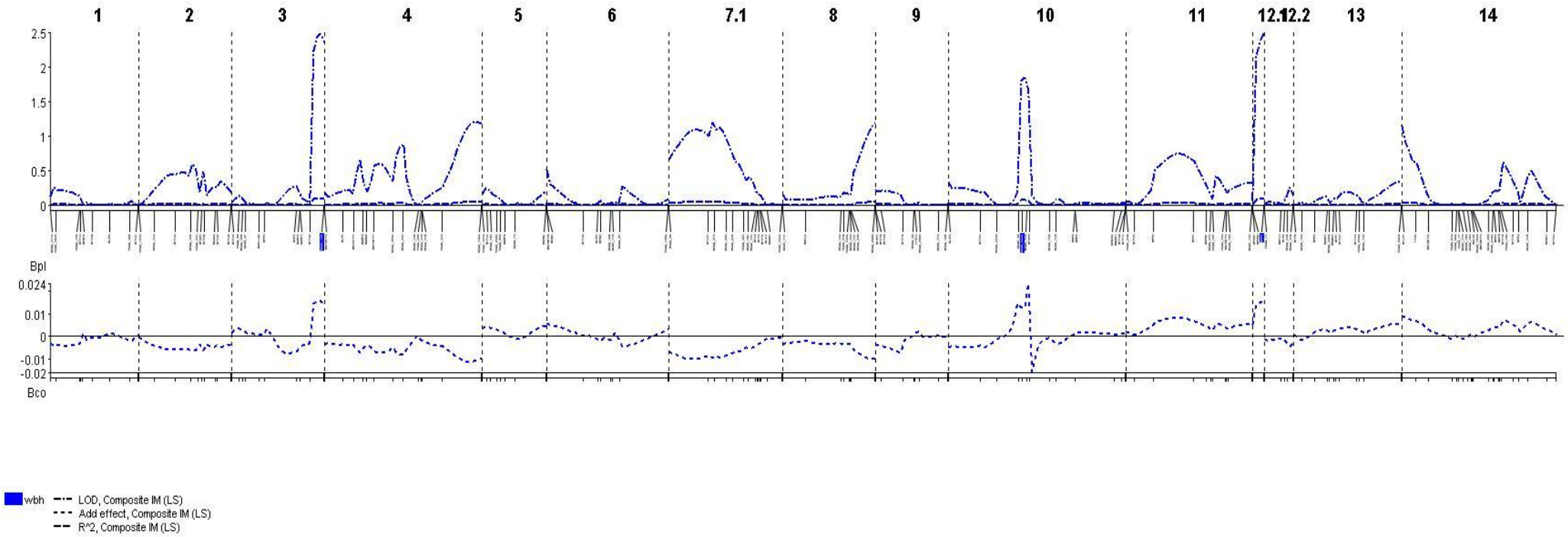

Figure 7.38: Depth of abaxial hypoderm scan of a test statistic scan of a test statistic for sCIM. A total of 162 markers covering the whole genome were used as background markers. LOD support intervals were calculated from the CIM results. Genome-wide threshold LOD values to declare a QTL to be significant were determined with 500 permutations. The threshold values obtained with 500 permutations were alpha 0.10 = 2.753, alpha 0.05 = 3.134 and alpha 0.01 = 4.506. Default measurements were used in QGENE to select cofactors. Selected background locus included BCAC_108 3 76.5, RCAG_254a 10 41.7, BHP1 12.1 6.9.

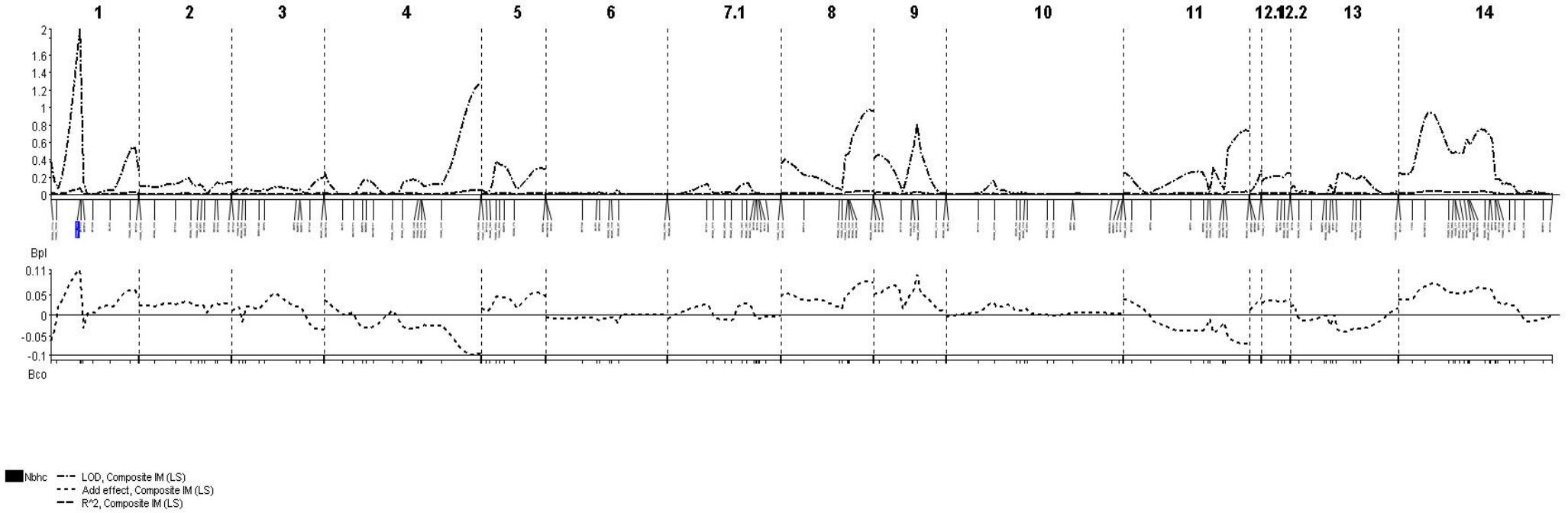

Figure 7.39: Number of abaxial hypoderm cells scan of a test statistic scan of a test statistic for sCIM. A total of 162 markers covering the whole genome were used as background markers. LOD support intervals were calculated from the CIM results. Genome-wide threshold LOD values to declare a QTL to be significant were determined with 500 permutations. The threshold values obtained with 500 permutations were alpha 0.10 = 2.862, alpha 0.05 = 3.541 and alpha 0.01 = 5.671. Default measurements were used in QGENE to select cofactors. Selected background locus included YCAA_120 1 18.0.

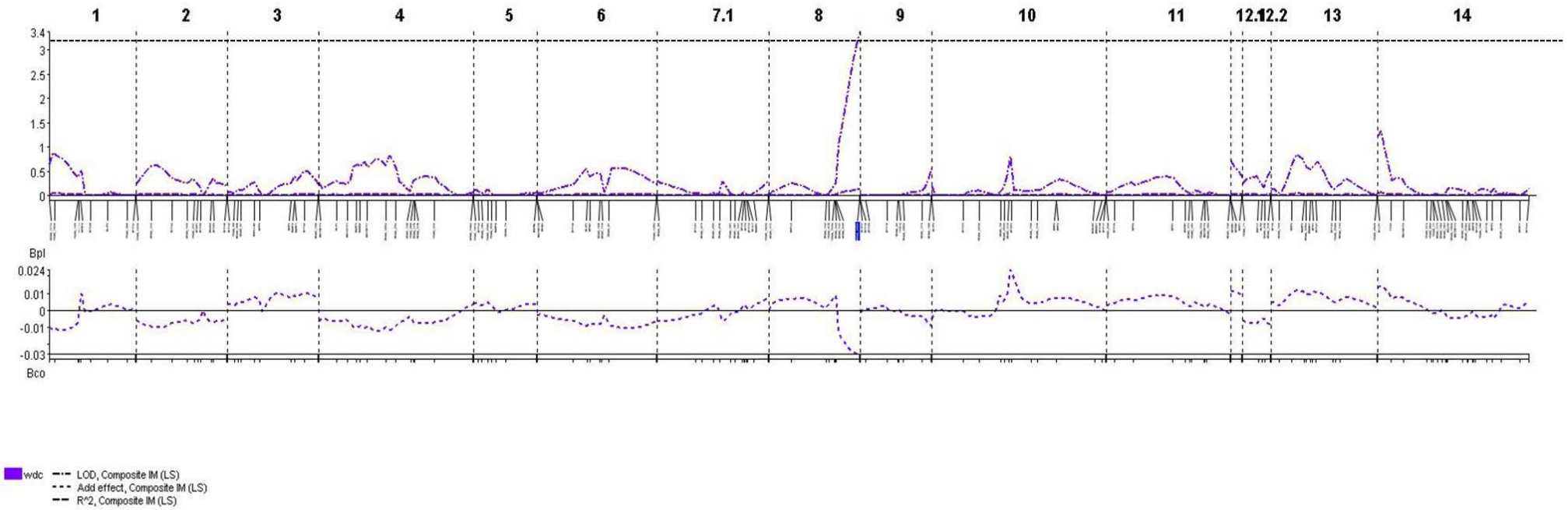

Figure 7.40: Width of adaxial cells scan of a test statistic scan of a test statistic for sCIM. A total of 162 markers covering the whole genome were used as background markers. LOD support intervals were calculated from the CIM results. Genome-wide threshold LOD values to declare a QTL to be significant were determined with 500 permutations. The threshold values obtained with 500 permutations were alpha 0.10 = 2.652, alpha 0.05 = 3.235 and alpha 0.01 = 4.057. Default measurements were used in QGENE to select cofactors. Selected background locus included RCAA_288b 8 70.9.

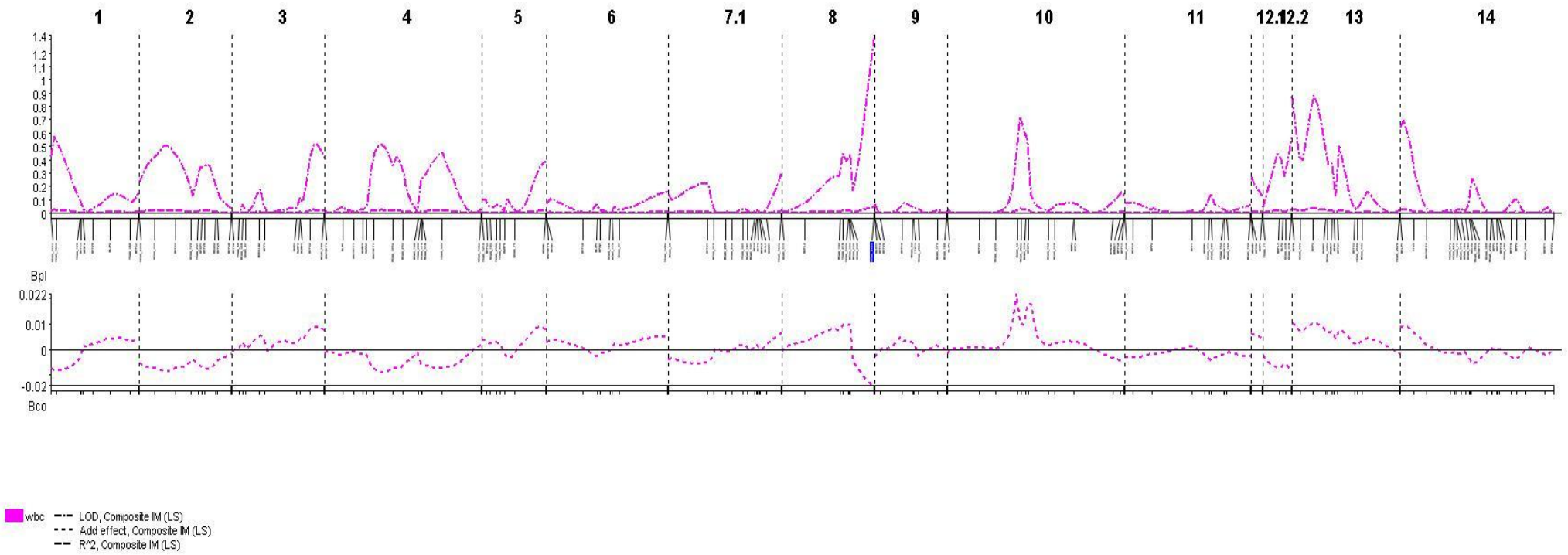

Figure 7.41: Widths of abaxial cells scan of a test statistic scan of a test statistic for sCIM. A total of 162 markers covering the whole genome were used as background markers. LOD support intervals were calculated from the CIM results. Genome-wide threshold LOD values to declare a QTL to be significant were determined with 500 permutations. The threshold values obtained with 500 permutations were alpha 0.10 = 2.815, alpha 0.05 = 3.169 and alpha 0.01 = 3.972. Default measurements were used in QGENE to select cofactors. Selected background locus included RCAA_288b 8 70.9.

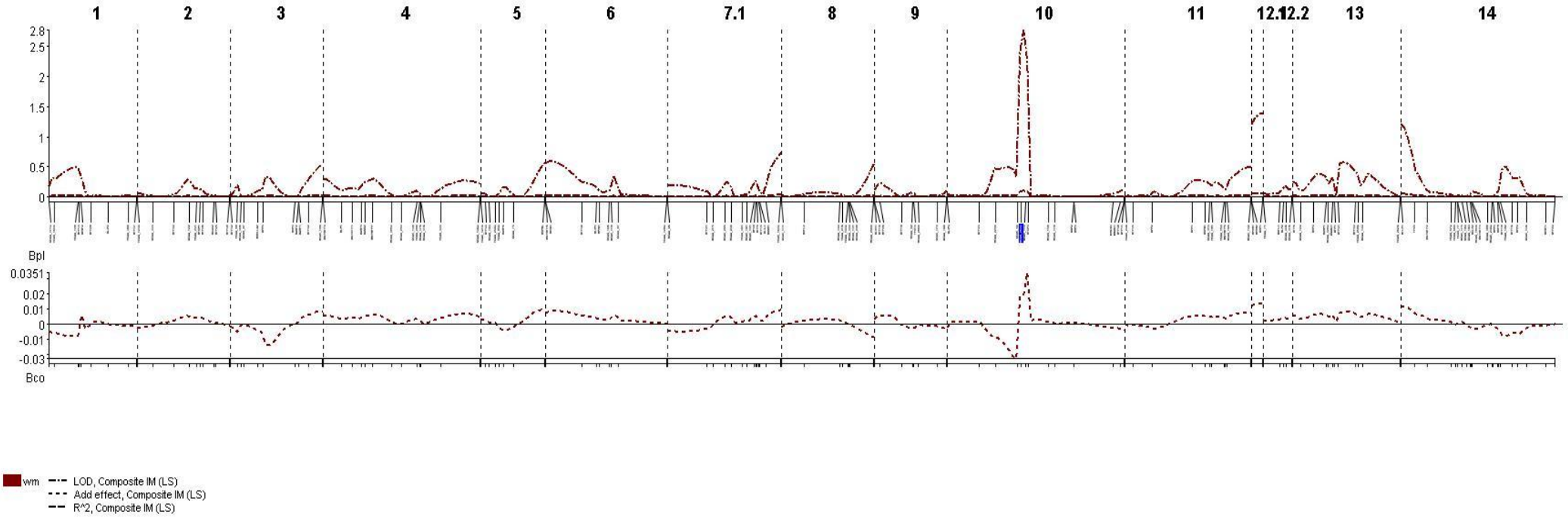

Figure 7.42: Width of mesophyll test statistic scan of a test statistic for sCIM. A total of 162 markers covering the whole genome were used as background markers. LOD support intervals were calculated from the CIM results. Genome-wide threshold LOD values to declare a QTL to be significant were determined with 500 permutations. The threshold values obtained with 500 permutations were alpha 0.10 = 2.66, alpha 0.05 = 3.099 and alpha 0.01 = 4.063. Default measurements were used in QGENE to select cofactors. Selected background locus included RCAA_305a 4 38.7.

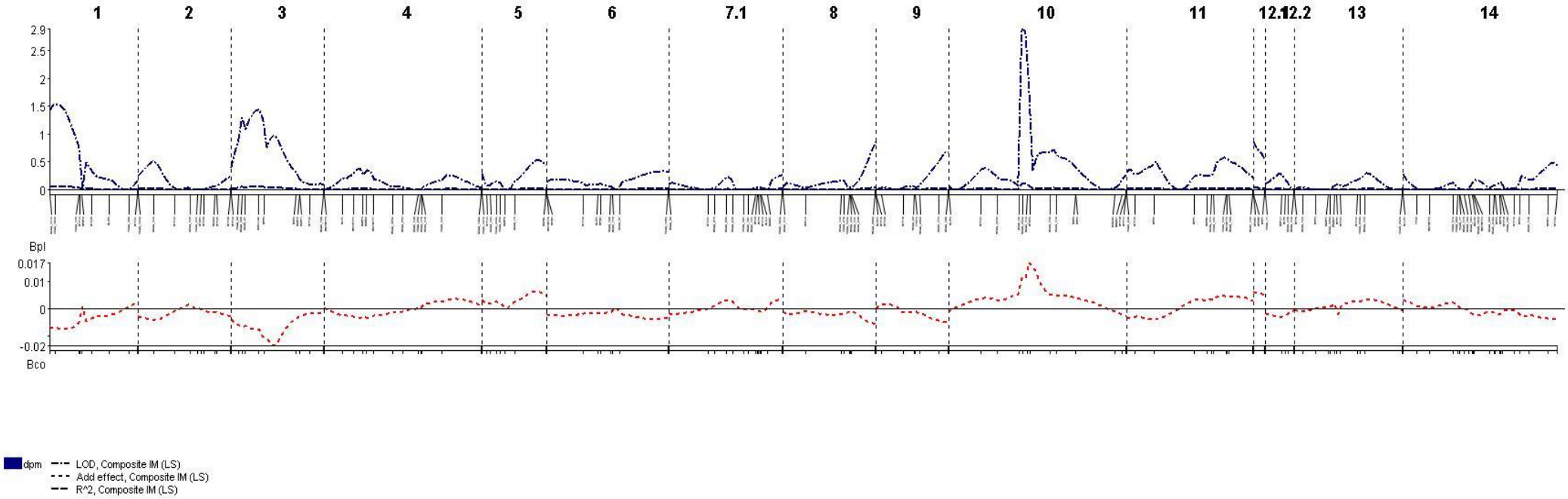

Figure 7.43: Depth of pallisade mesophyll test statistic scan of a test statistic for sCIM. A total of 162 markers covering the whole genome were used as background markers. LOD support intervals were calculated from the CIM results. Genome-wide threshold LOD values to declare a QTL to be significant were determined with 500 permutations. The threshold values obtained with 500 permutations were alpha 0.10 = 2.52, alpha 0.05 = 2.802 and alpha 0.01 = 3.555. Default measurements were used in QGENE to select cofactors. Selected background locus included (Redo).

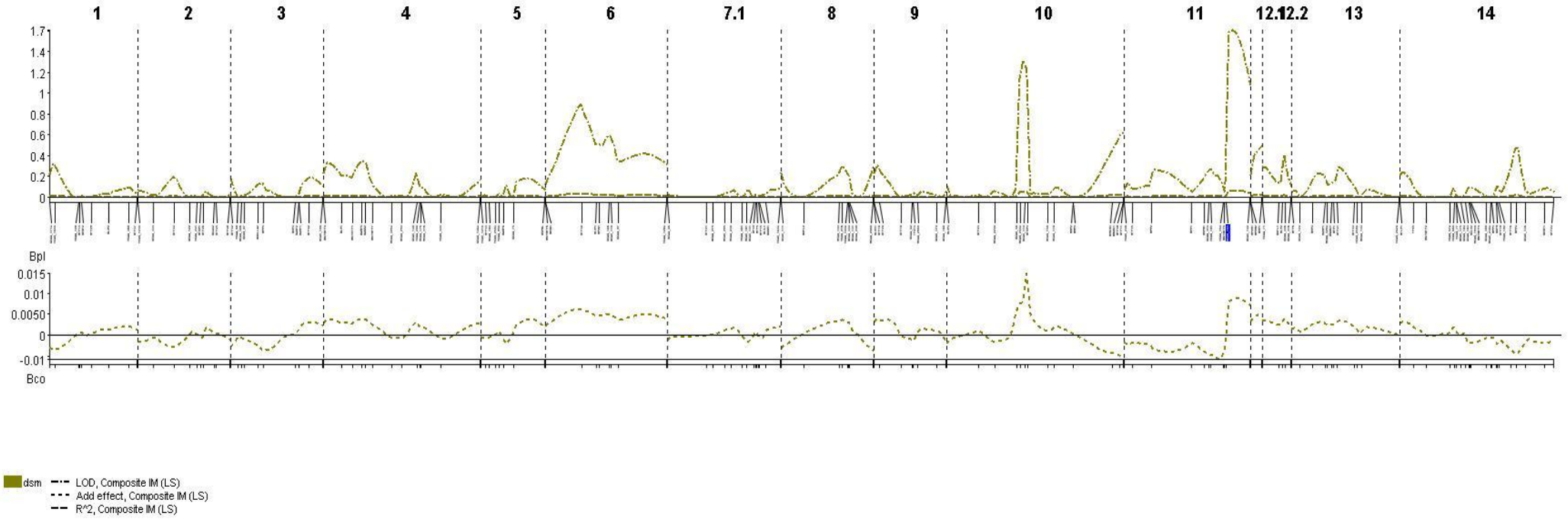

Figure 7.44: Depth of spongy mesophyll test statistic scan of a test statistic for sCIM. A total of 162 markers covering the whole genome were used as background markers. LOD support intervals were calculated from the CIM results. Genome-wide threshold LOD values to declare a QTL to be significant were determined with 500 permutations. The threshold values obtained with 500 permutations were alpha 0.10 = 2.649, alpha 0.05 = 2.99 and alpha 0.01 = 4.326. Default measurements were used in QGENE to select cofactors. Selected background locus included RCAG_165 11 71.2.

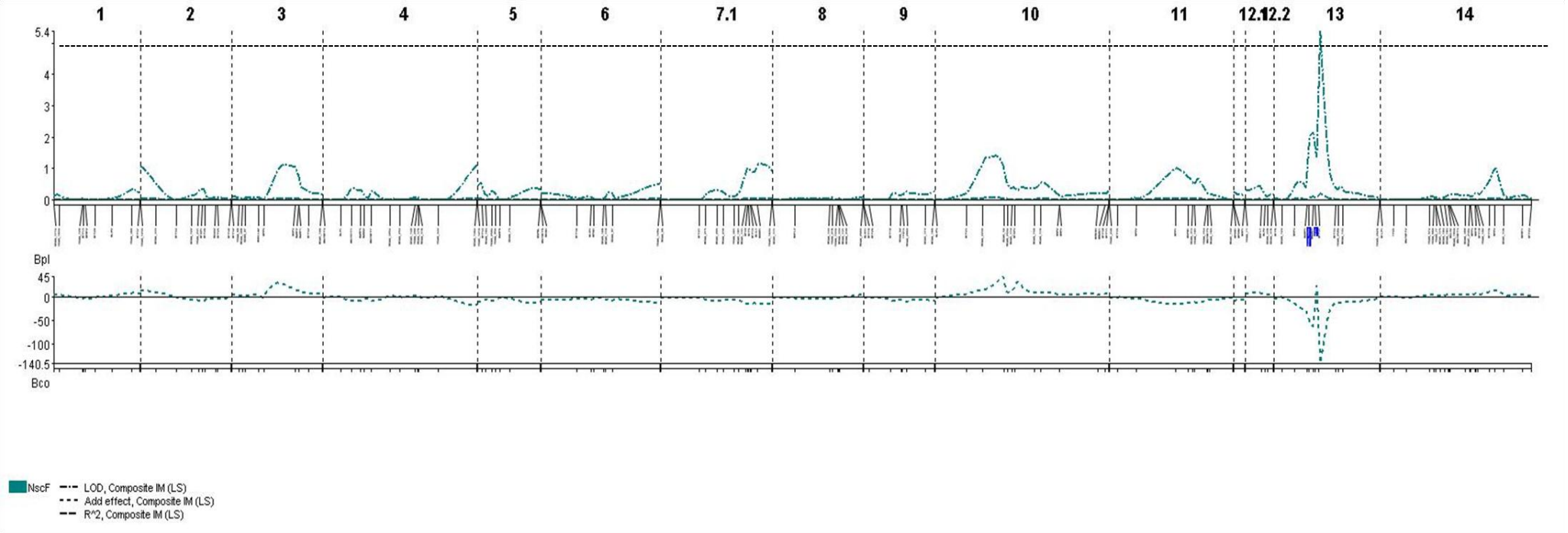

Figure 7.45: Number of stomatal clusters per mm^2 of leaf test statistic scan of a test statistic for sCIM. A total of 162 markers covering the whole genome were used as background markers. LOD support intervals were calculated from the CIM results. Genome-wide threshold LOD values to declare a QTL to be significant were determined with 500 permutations. The threshold values obtained with 500 permutations were alpha 0.10 = 2.631, alpha 0.05 = 3.167 and alpha 0.01 = 4.709. Default measurements were used in QGENE to select cofactors. Selected background locus included RCAA_328a 13 36.6, BFP1 13 40.1.

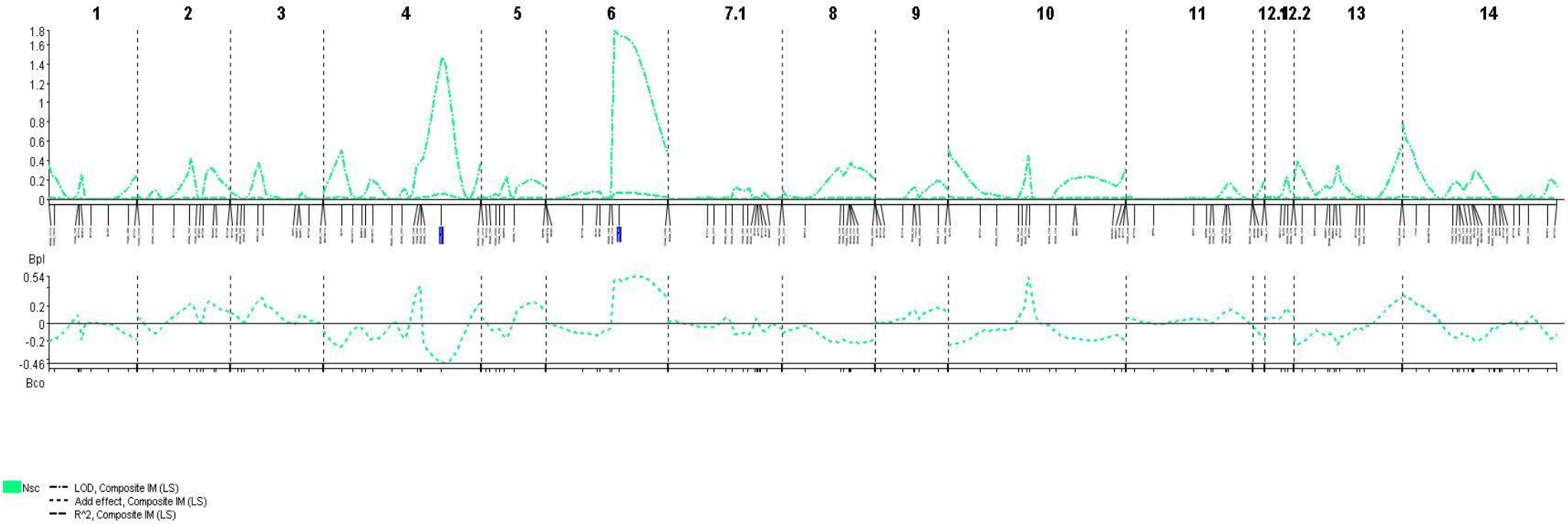

Figure 7.46: Number of stomata per cluster of leaf scan of a test statistic for sCIM. A total of 162 markers covering the whole genome were used as background markers. LOD support intervals were calculated from the CIM results. Genome-wide threshold LOD values to declare a QTL to be significant were determined with 500 permutations. The threshold values obtained with 500 permutations were alpha 0.10 = 2.746, alpha 0.05 = 3.159 and alpha 0.01 = 4.288. Default measurements were used in QGENE to select cofactors. Selected background locus included YCAG_344 4 66.3, GCAA_97 6 41.5.

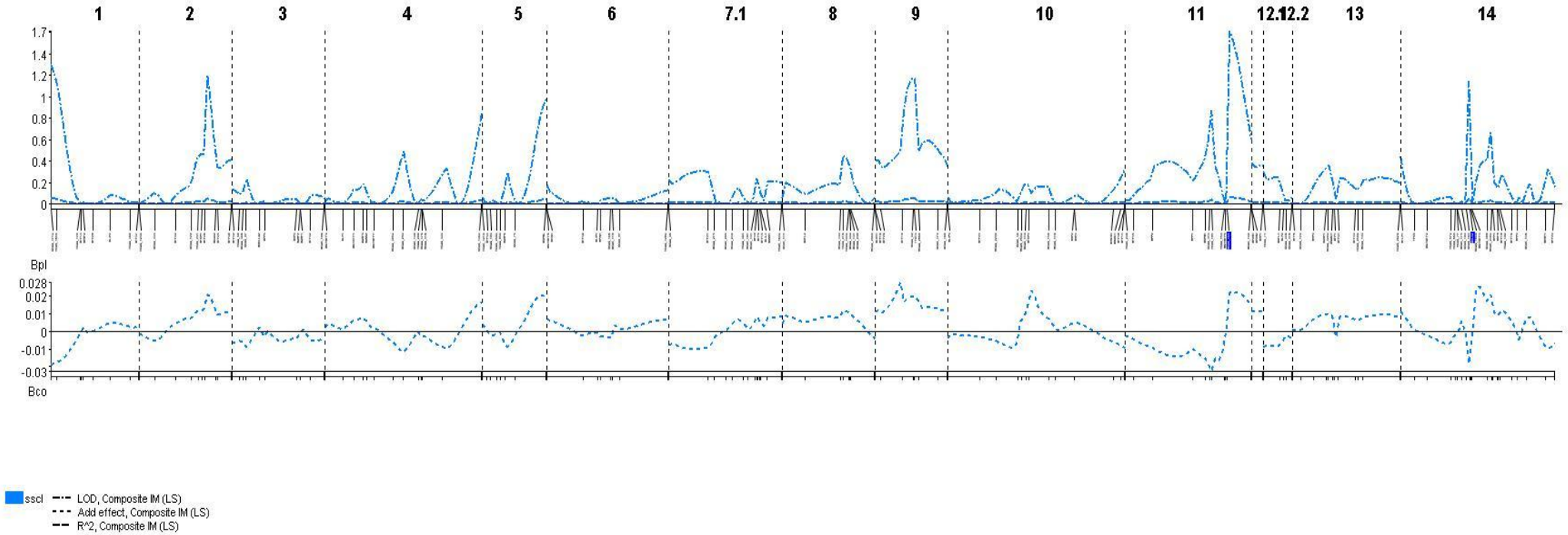

Figure 7.47: Sub stomatal cluster length test statistic scan of a test statistic for sCIM. A total of 162 markers covering the whole genome were used as background markers. LOD support intervals were calculated from the CIM results. Genome-wide threshold LOD values to declare a QTL to be significant were determined with 500 permutations. The threshold values obtained with 500 permutations were alpha 0.10 = 2.668, alpha 0.05 = 3.029 and alpha 0.01 = 4.259. Default measurements were used in QGENE to select cofactors. Selected background locus included RCAG_165 11 71.2, BCLV2 14 39.3.

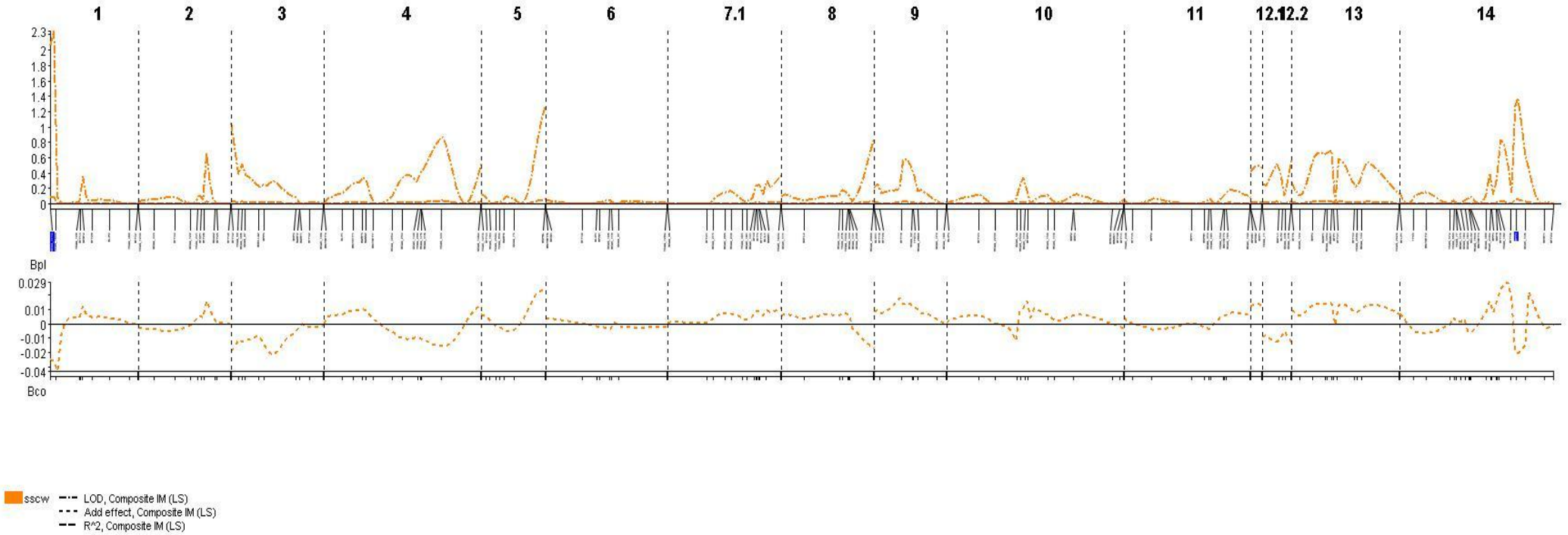

Figure 7.48: Sub stomatal cluster width test statistic scan of a test statistic for sCIM. A total of 162 markers covering the whole genome were used as background markers. LOD support intervals were calculated from the CIM results. Genome-wide threshold LOD values to declare a QTL to be significant were determined with 500 permutations. The threshold values obtained with 500 permutations were alpha 0.10 = 2.781, alpha 0.05 = 3.255 and alpha 0.01 = 4.08. Default measurements were used in QGENE to select cofactors. Selected background locus included alpha GCAA_171a 1 1.2 and BPP4 14 65.6.

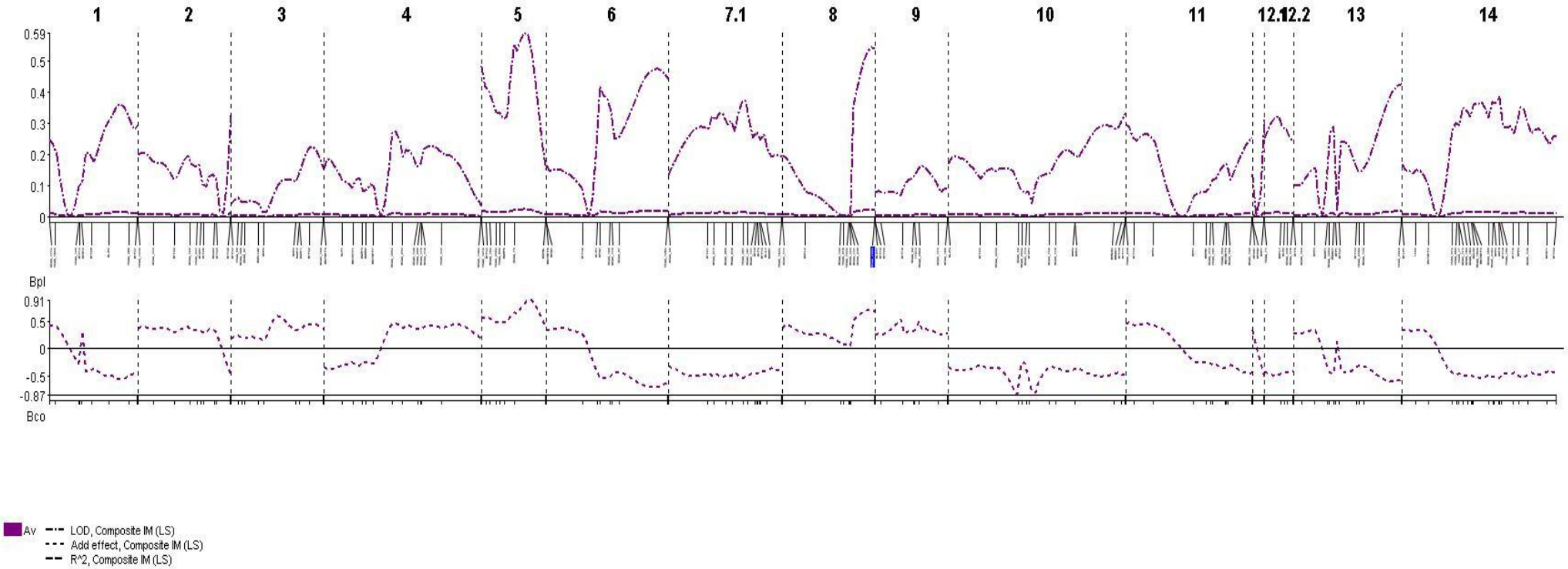

Figure 7.49: Average number of stomata per cluster scan of a test statistic for sCIM. A total of 162 markers covering the whole genome were used as background markers. LOD support intervals were calculated from the CIM results. Genome-wide threshold LOD values to declare a QTL to be significant were determined with 500 permutations. The threshold values obtained with 500 permutations were alpha 0.10 = 3.882 alpha 0.05 = 6.43 and alpha 0.01 = 20.427. Default measurements were used in QGENE to select cofactors. Selected background locus included RCAA_288b 8 70.9.

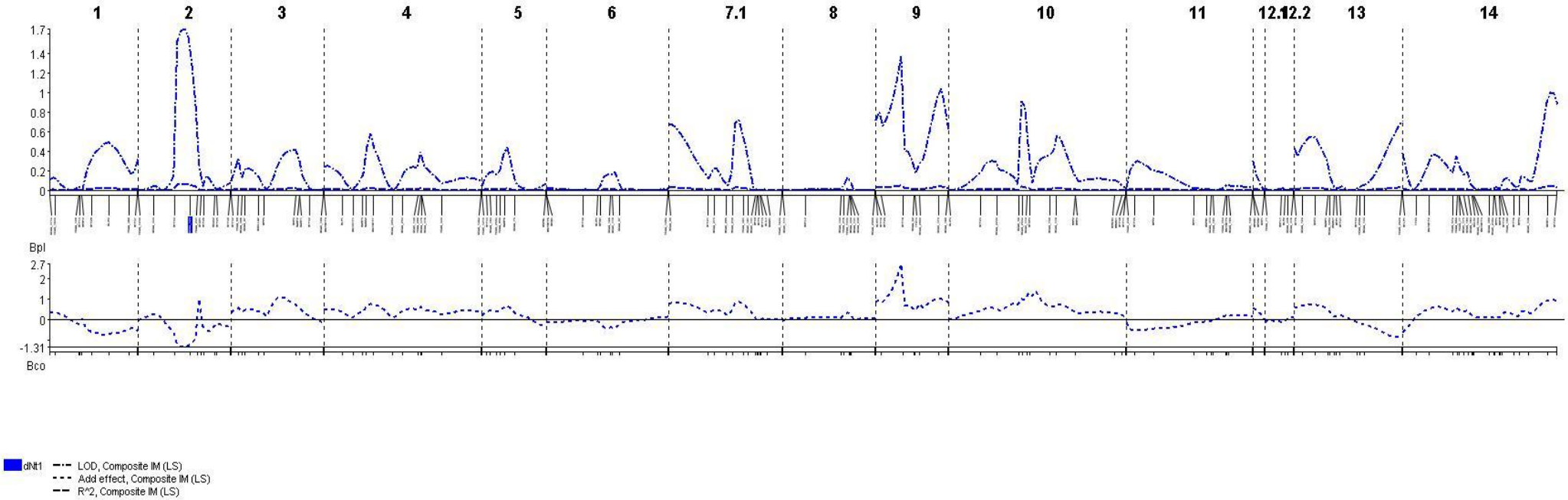

Figure 7.50: Adaxial number of whiplash trichome scan of a test statistic for sCIM. A total of 162 markers covering the whole genome were used as background markers. LOD support intervals were calculated from the CIM results. Genome-wide threshold LOD values to declare a QTL to be significant were determined with 500 permutations. The threshold values obtained with 500 permutations were alpha 0.10 = 3.222, alpha 0.05 = 3.954 and alpha 0.01 = 5.135. Default measurements were used in QGENE to select cofactors. Selected background locus included GCAA_122 2 29.6.

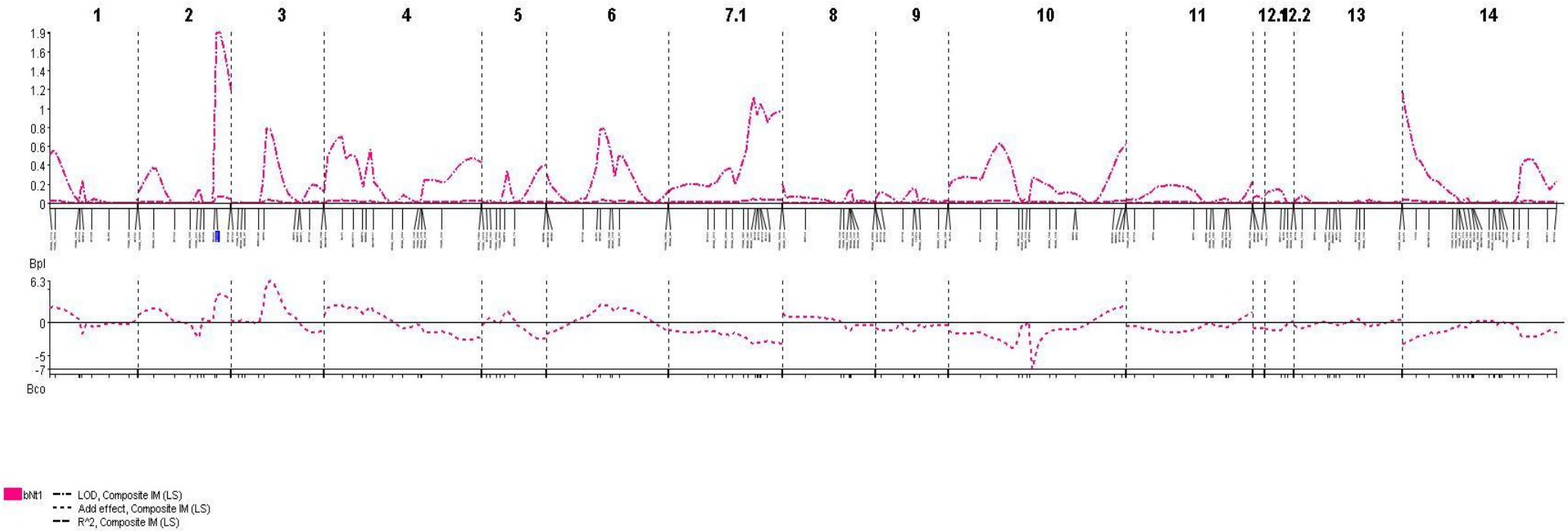

Figure 7.51: Abaxial number of whiplash trichome scan of a test statistic for sCIM. A total of 162 markers covering the whole genome were used as background markers. LOD support intervals were calculated from the CIM results. Genome-wide threshold LOD values to declare a QTL to be significant were determined with 500 permutations. The threshold values obtained with 500 permutations were alpha 0.10 = 2.828, alpha 0.05 = 3.454 and alpha 0.01 = 4.221. Default measurements were used in QGENE to select cofactors. Selected background locus included BTF25 2 44.5.

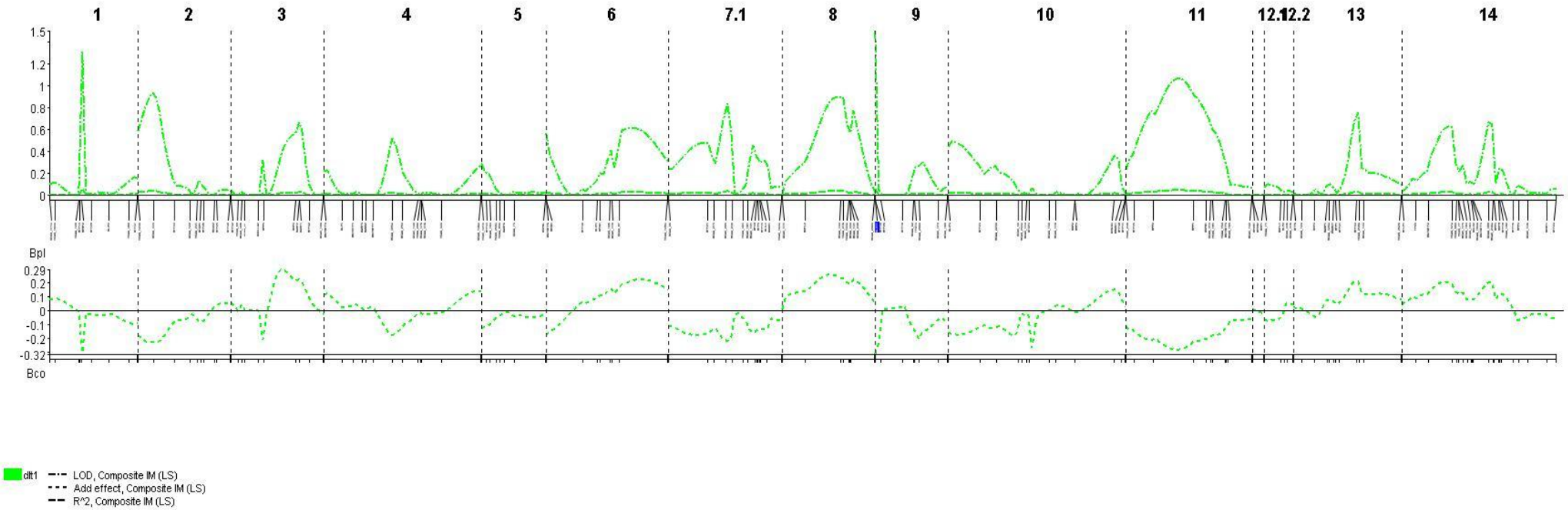

Figure 7.52: Adaxial length of whiplash trichome scan of a test statistic for sCIM. A total of 162 markers covering the whole genome were used as background markers. LOD support intervals were calculated from the CIM results. Genome-wide threshold LOD values to declare a QTL to be significant were determined with 500 permutations. The threshold values obtained with 500 permutations were alpha 0.10 = 2.859, alpha 0.05 = 3.484 and alpha 0.01 = 4.811. Default measurements were used in QGENE to select cofactors. Selected background locus included BLHY1 9 0.0.

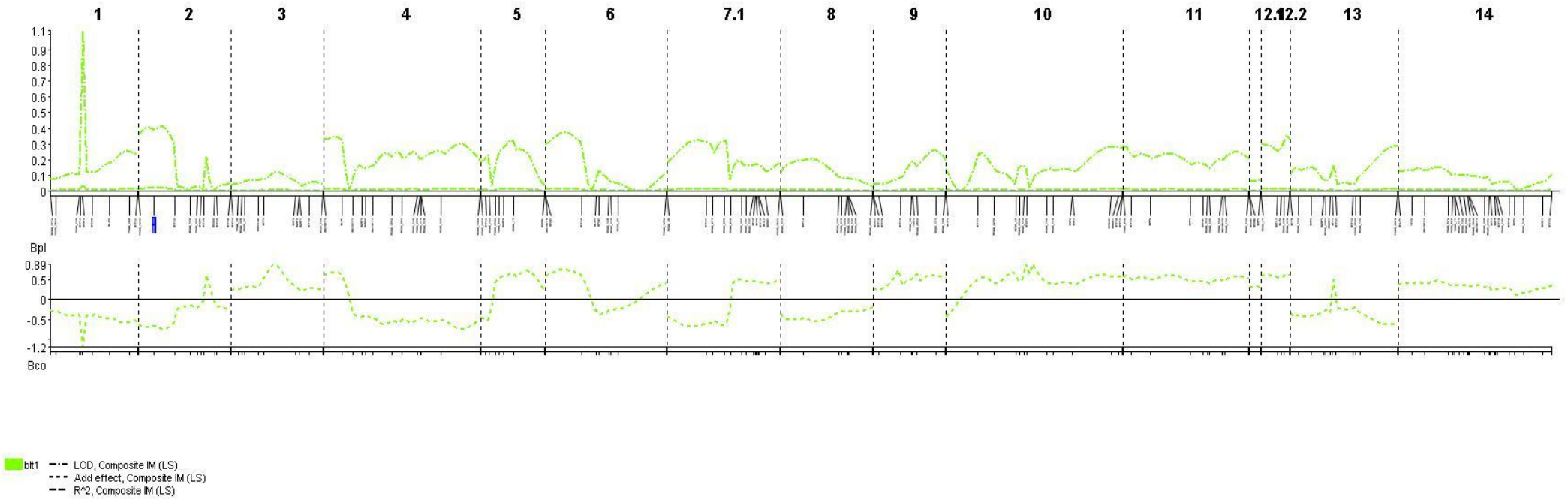

Figure 7.53: Abaxial length of glandular trichomes scan of a test statistic for sCIM. A total of 162 markers covering the whole genome were used as background markers. LOD support intervals were calculated from the CIM results. Genome-wide threshold LOD values to declare a QTL to be significant were determined with 500 permutations. The threshold values obtained with 500 permutations were alpha 0.10 = 3.006, alpha 0.05 = 3.948 and alpha 0.01 = 5.345. Default measurements were used in QGENE to select cofactors. Selected background locus included BCAA_180 9 40.8, BLP2 12.2 17.1.

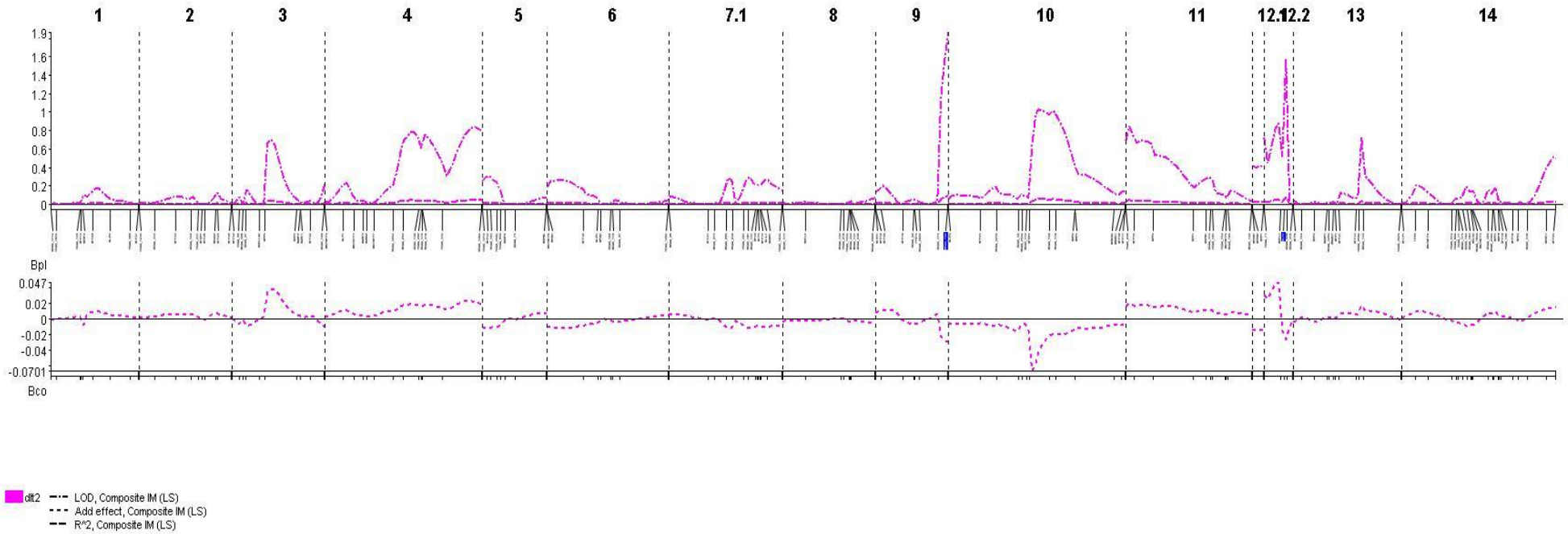

Figure 7.54: Abaxial length of glandular trichome scan of a test statistic for sCIM. A total of 162 markers covering the whole genome were used as background markers. LOD support intervals were calculated from the CIM results. Genome-wide threshold LOD values to declare a QTL to be significant were determined with 500 permutations. The threshold values obtained with 500 permutations were alpha 0.10 = 3.248, alpha 0.05 = 4.268 and alpha 0.01 = 11.213. Default measurements were used in QGENE to select cofactors. Selected background locus included GCAA_333 2 9.0.

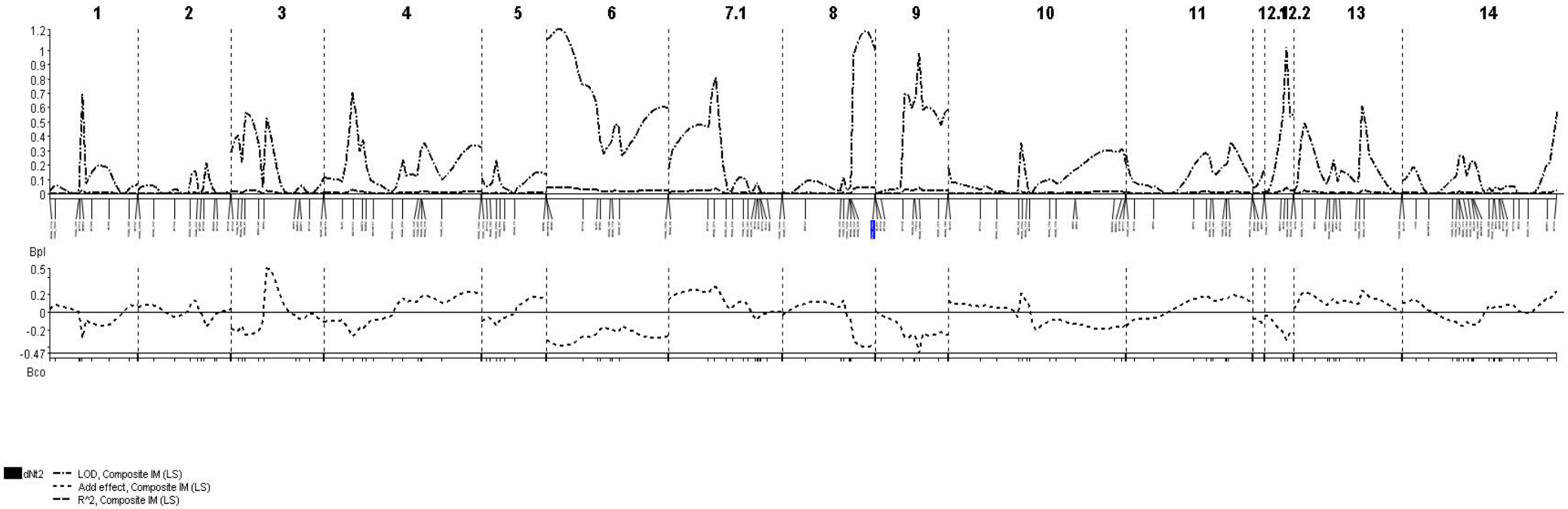

Figure 7.55: Adaxial number of glandular trichome scan of a test statistic for sCIM. A total of 162 markers covering the whole genome were used as background markers. LOD support intervals were calculated from the CIM results. Genome-wide threshold LOD values to declare a QTL to be significant were determined with 500 permutations. The threshold values obtained with 500 permutations were alpha 0.10 = 2.745, alpha 0.05 = 3.113 and alpha 0.01 = 3.914. Default measurements were used in QGENE to select cofactors. Selected background locus included RCAA_288b 8 70.9.

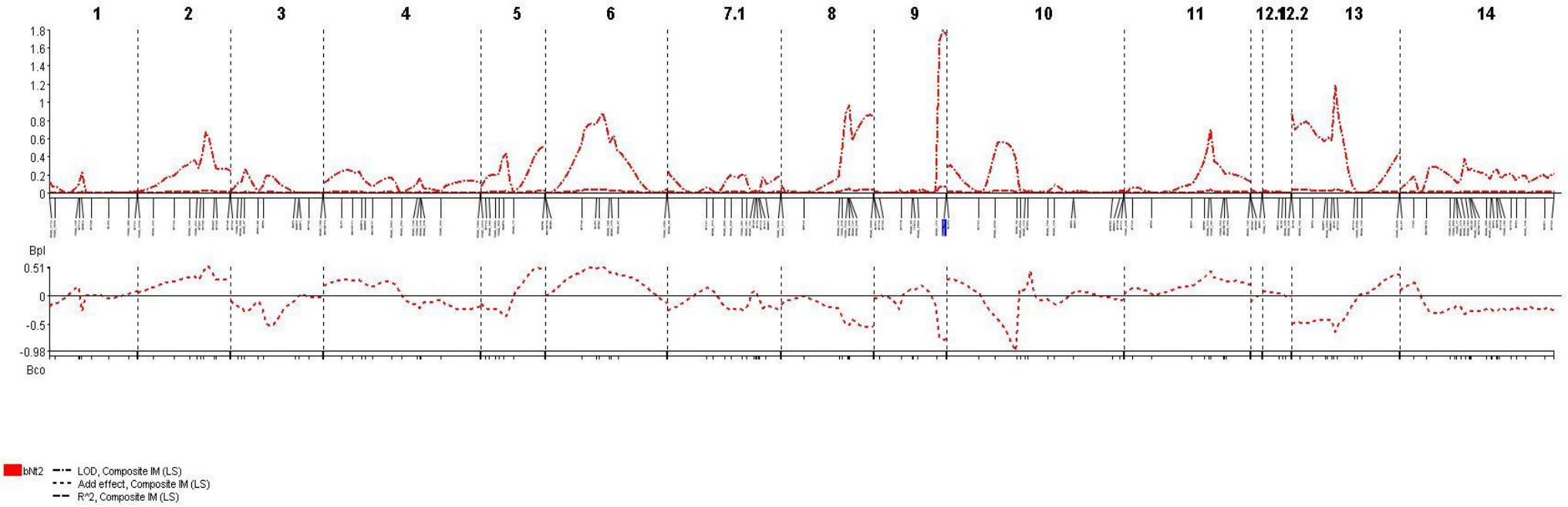

Figure 7.56: Abaxial number of glandular trichomes scan of a test statistic for sCIM. A total of 162 markers covering the whole genome were used as background markers. LOD support intervals were calculated from the CIM results. Genome-wide threshold LOD values to declare a QTL to be significant were determined with 500 permutations. The threshold values obtained with 500 permutations were alpha 0.10 = 2.747, alpha 0.05 = 3.195 and alpha 0.01 = 3.848. Default measurements were used in QGENE to select cofactors. Selected background locus included BCAA_180 9 40.8

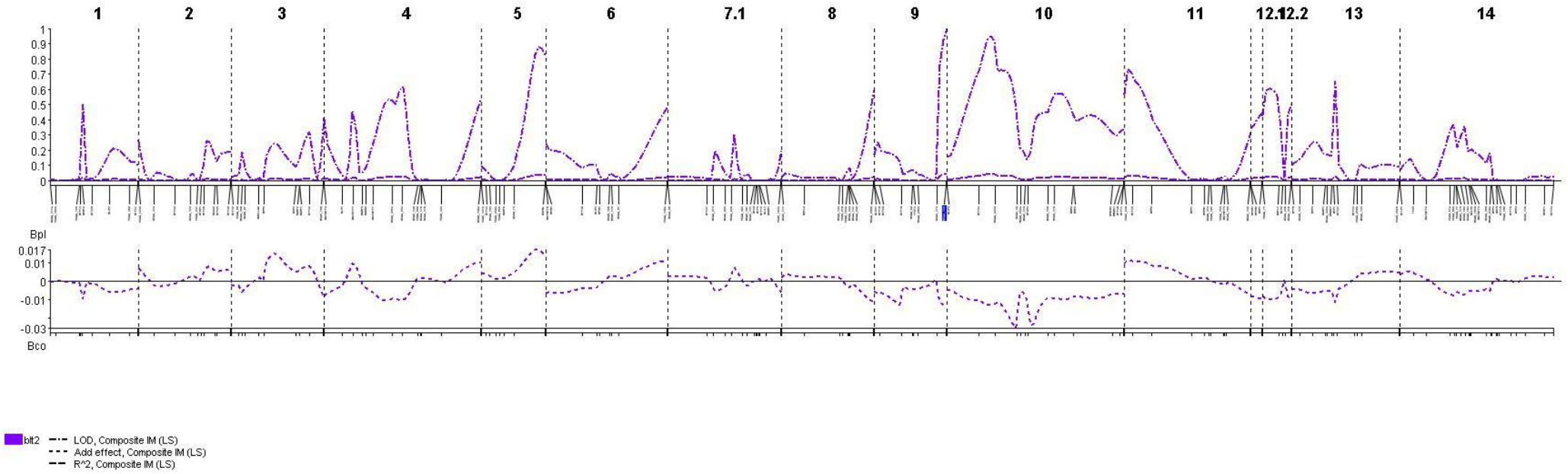

Figure 7.57: Abaxial length of glandular trichome scan of a test statistic for sCIM. A total of 162 markers covering the whole genome were used as background markers. LOD support intervals were calculated from the CIM results. Genome-wide threshold LOD values to declare a QTL to be significant were determined with 500 permutations. The threshold values obtained with 500 permutations were alpha 0.10 = 2.862, alpha 0.05 = 3.558 and alpha 0.01 = 6.923. Default measurements were used in QGENE to select cofactors. Selected background locus included BCAA_180 9 40.8.

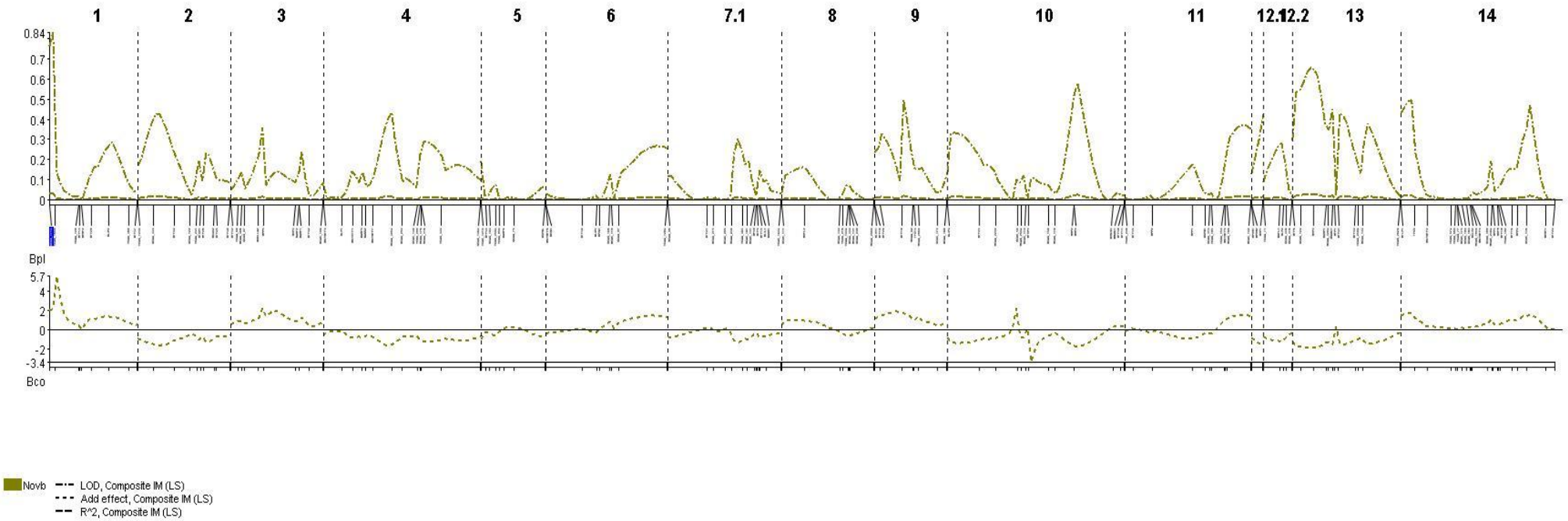

Figure 7.58: Number of outer vascular bundle scan of a test statistic for sCIM. A total of 162 markers covering the whole genome were used as background markers. LOD support intervals were calculated from the CIM results. Genome-wide threshold LOD values to declare a QTL to be significant were determined with 500 permutations. The threshold values obtained with 500 permutations were alpha 0.10 = 2.748, alpha 0.05 = 3.259 and alpha 0.01 = 4.377. Default measurements were used in QGENE to select cofactors. Selected background locus included GCAA_171a 1 1.2.

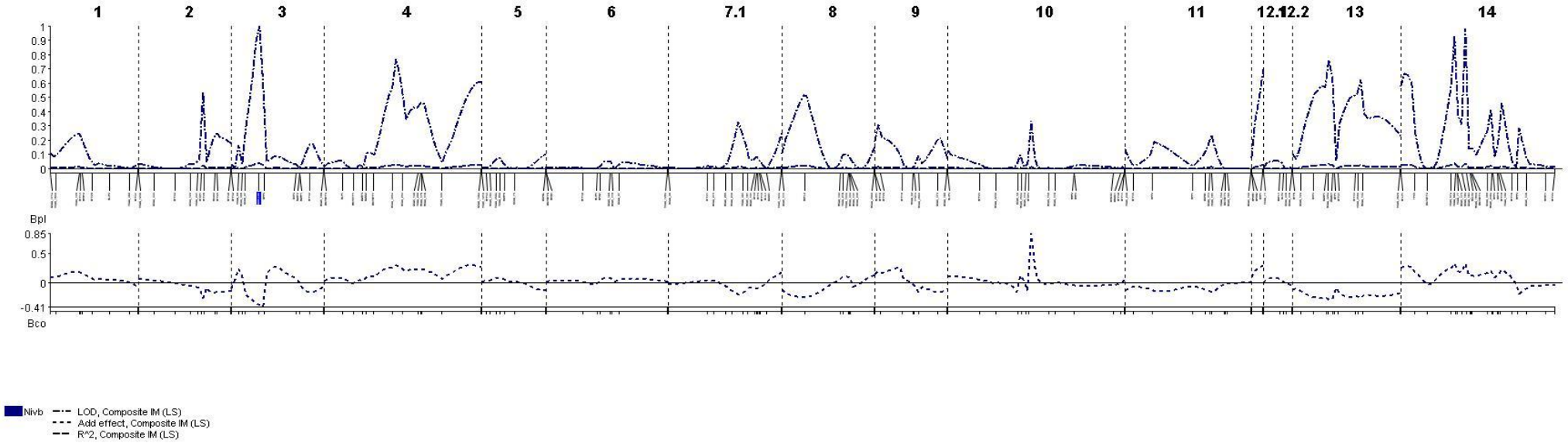

Figure 7.59: Number of inner vascular bundle scan of a test statistic for sCIM. A total of 162 markers covering the whole genome were used as background markers. LOD support intervals were calculated from the CIM results. Genome-wide threshold LOD values to declare a QTL to be significant were determined with 500 permutations. The threshold values obtained with 500 permutations were alpha 0.10 = 2.69, alpha 0.05 = 3.293 and alpha 0.01 = 4.159. Default measurements were used in QGENE to select cofactors. Selected background locus included BDELLA2 3 39.9.

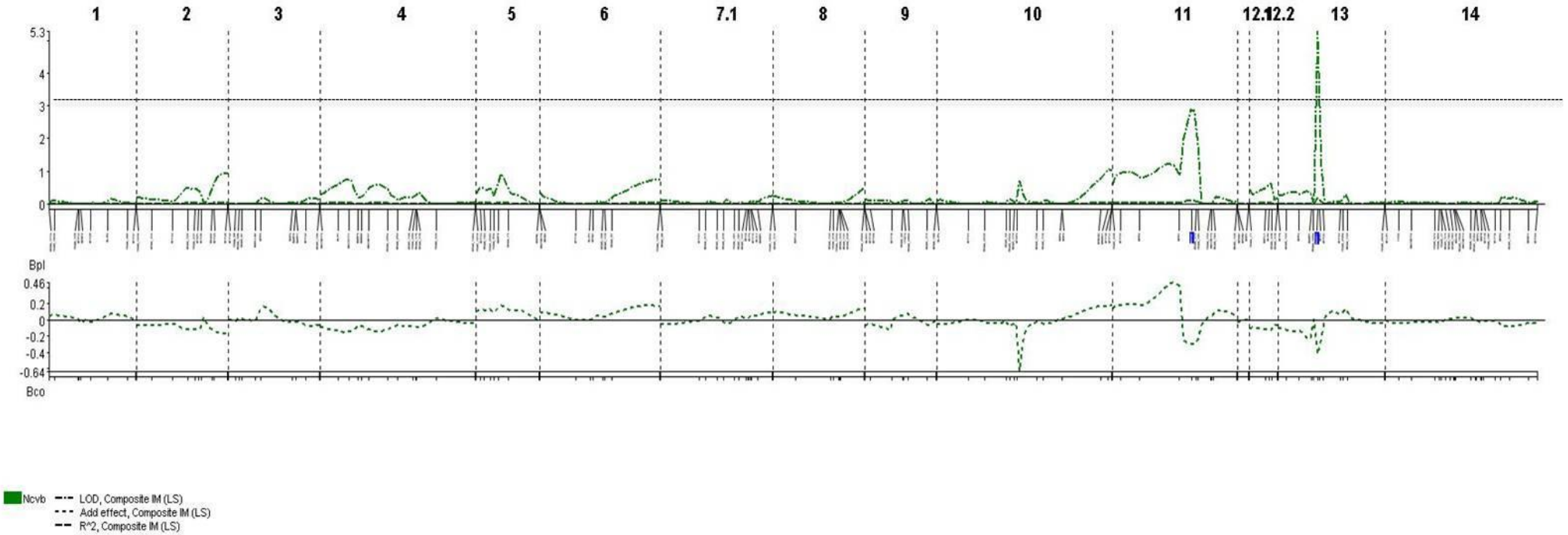

Figure 7.60: Number of central vascular bundle scan of a test statistic for sCIM. A total of 162 markers covering the whole genome were used as background markers. LOD support intervals were calculated from the CIM results. Genome-wide threshold LOD values to declare a QTL to be significant were determined with 500 permutations. The threshold values obtained with 500 permutations were alpha 0.10 = 2.663, alpha 0.05 = 3.258 and alpha 0.01 = 4.457. Default measurements were used in QGENE to select cofactors. Selected background locus included BSPB1 11 58.9, BNAM1 13 39.3.

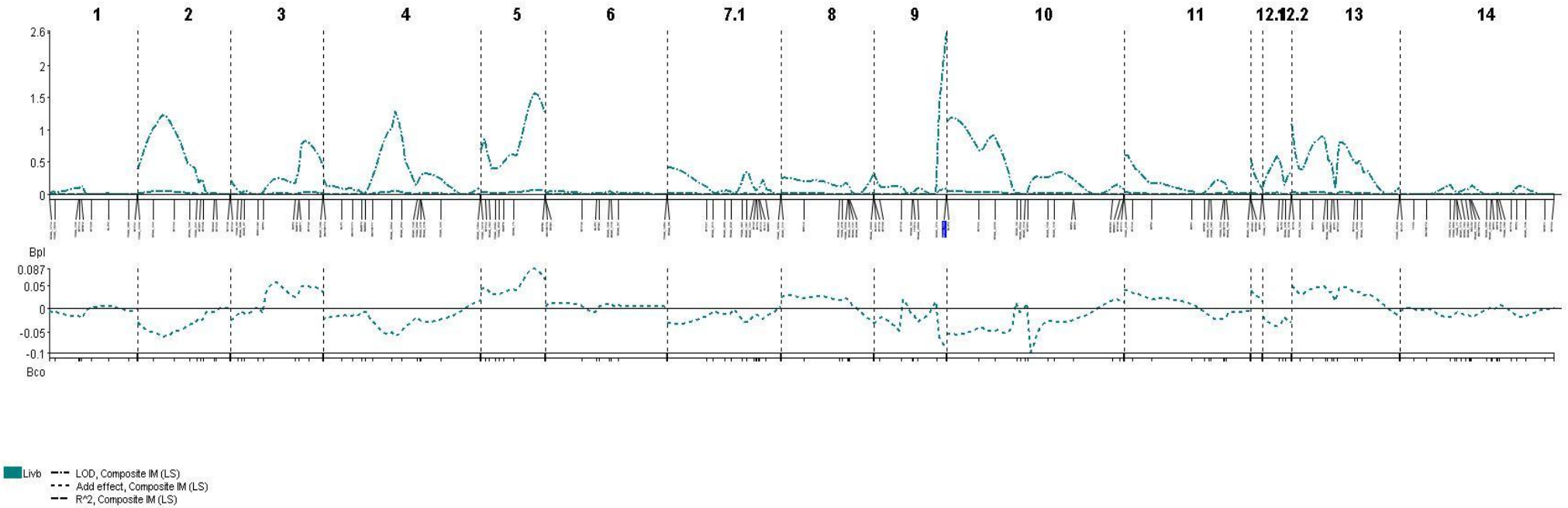

Figure 7.61: Length of inner vascular bundle scan of a test statistic for sCIM. A total of 162 markers covering the whole genome were used as background markers. LOD support intervals were calculated from the CIM results. Genome-wide threshold LOD values to declare a QTL to be significant were determined with 500 permutations. The threshold values obtained with 500 permutations were alpha 0.10 = 2.822, alpha 0.05 = 3.45 and alpha 0.01 = 4.539. Default measurements were used in QGENE to select cofactors. Selected background locus included BCAA_180 9 40.8.

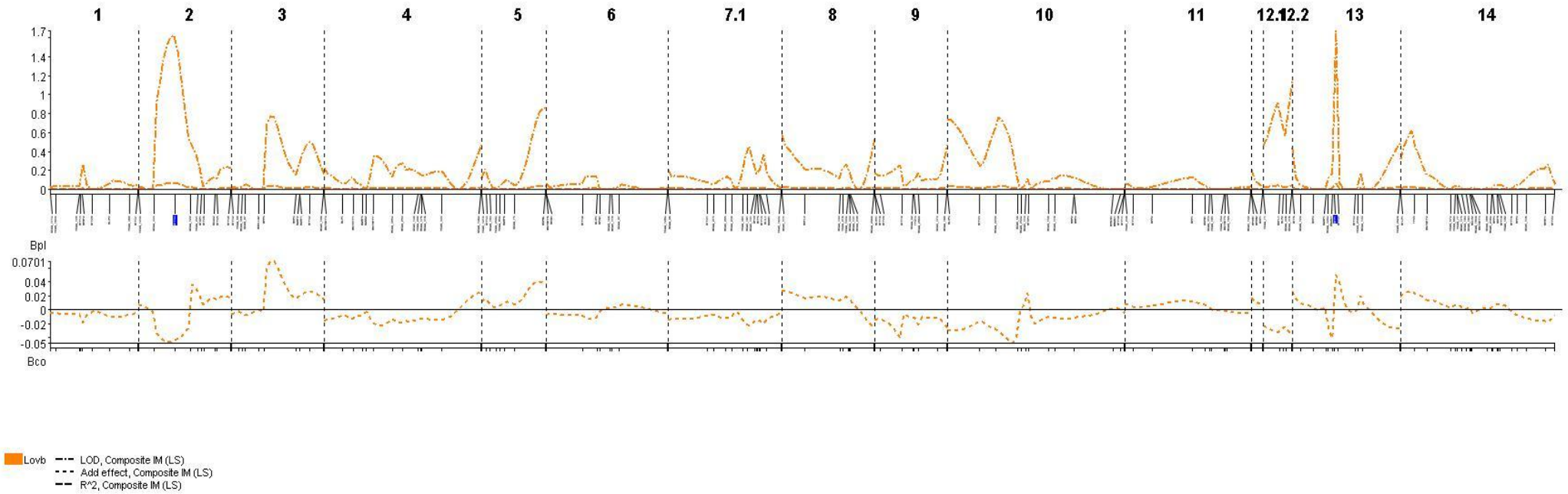

Figure 7.62: Length of outer vascular bundle scan of a test statistic for sCIM. A total of 162 markers covering the whole genome were used as background markers. LOD support intervals were calculated from the CIM results. Genome-wide threshold LOD values to declare a QTL to be significant were determined with 500 permutations. The threshold values obtained with 500 permutations were alpha 0.10 = 2.746 alpha 0.05 = 3.286 and alpha 0.01 = 4.4. Default measurements were used in QGENE to select cofactors. Selected background locus included BTF44 2 20.9, BFP1 13 40.1.

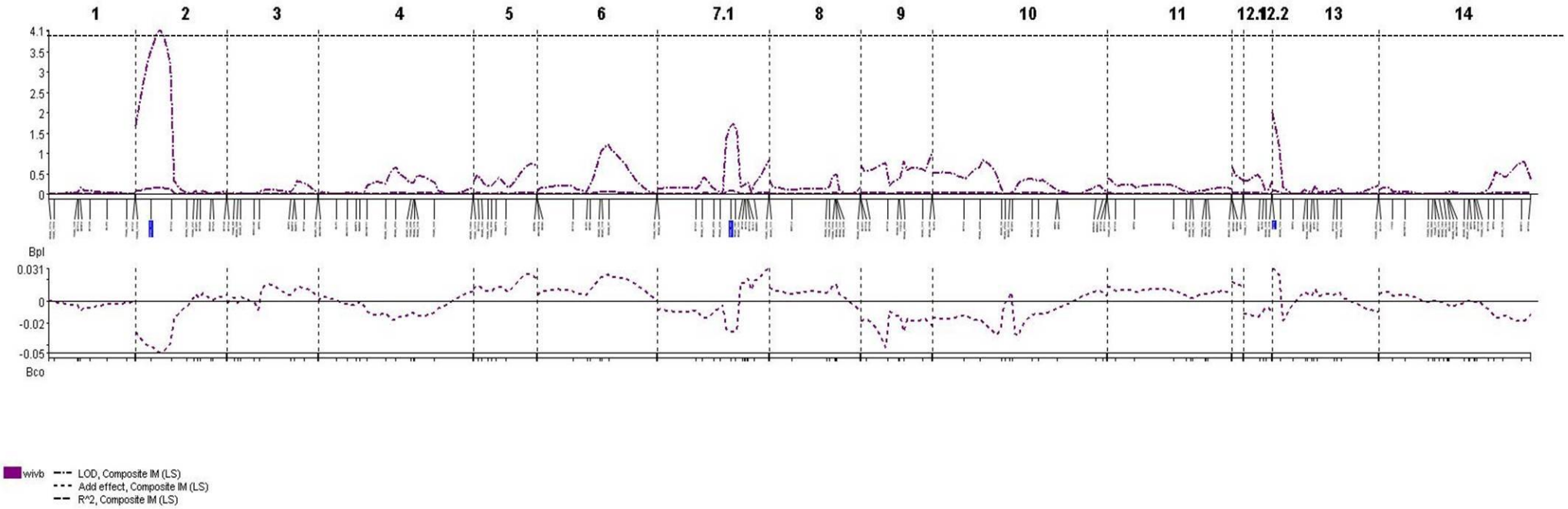

Figure 7.63: Width of inner vascular bundle scan of a test statistic for sCIM. A total of 162 markers covering the whole genome were used as background markers. LOD support intervals were calculated from the CIM results. Genome-wide threshold LOD values to declare a QTL to be significant were determined with 500 permutations. The threshold values obtained with 500 permutations were alpha 0.10 = 2.792, alpha 0.05 = 3.206 and alpha 0.01 = 4.186. Default measurements were used in QGENE to select cofactors. Selected background locus included GCAA_333 2 9.0, YCAA_381 7.1 42.7, BTF6 13 16.0.

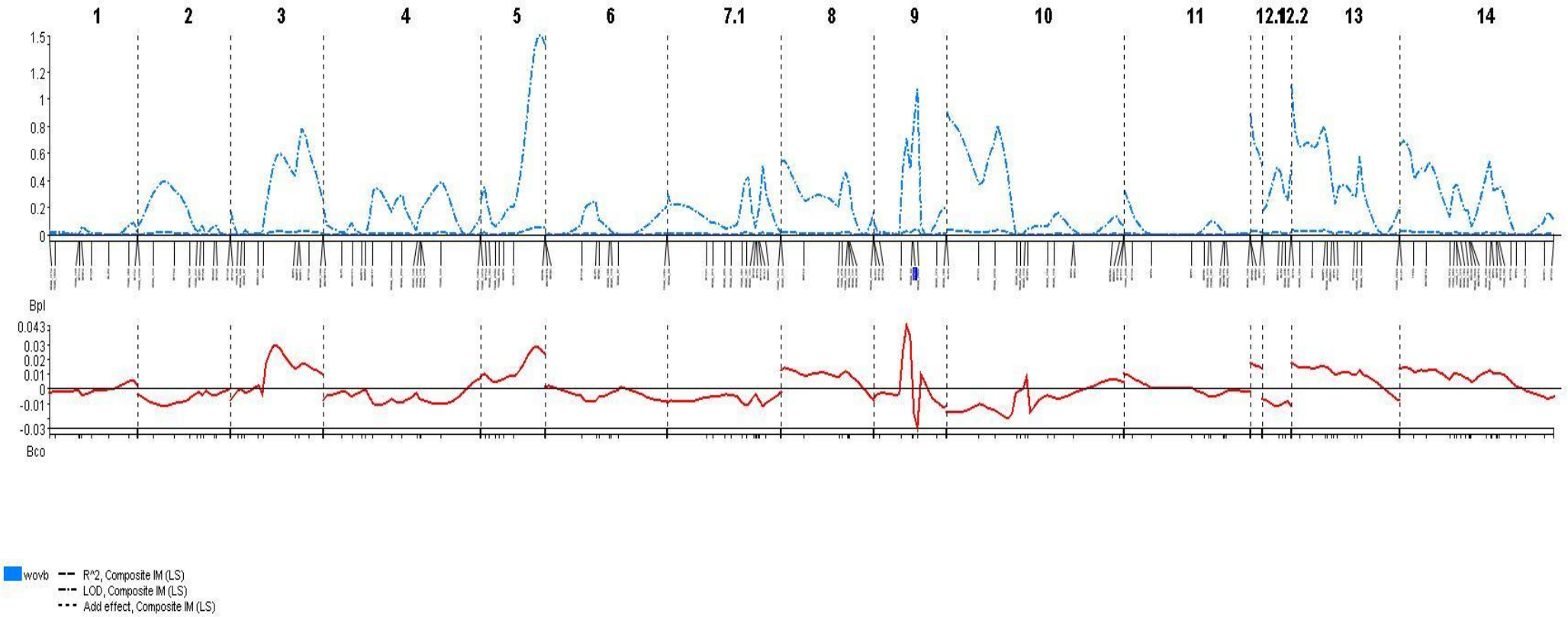

Figure 7.64: Width of outer vascular bundle scan of a test statistic for sCIM. A total of 162 markers covering the whole genome were used as background markers. LOD support intervals were calculated from the CIM results. Genome-wide threshold LOD values to declare a QTL to be significant were determined with 500 permutations. The threshold values obtained with 500 permutations were alpha 0.10 = 0.104, alpha 0.05 = 0.12 and alpha 0.01 = 0.152. Default measurements were used in QGENE to select cofactors. Selected background locus included TFC13 9 22.6.

Table 7.1: Composite interval mapping and MIM-GLZ mapping results. Markers associated with significant QTLs in the CIM analysis were regressed against untransformed trait values, R2 values from this analysis are used to estimate the percentage of phenotypic variation explained by the QTLs detected in the CIM and MIM-GLZanalysis.

Analysis	Trait (units)	Linkage group	Marker Interval	CIM (Add effect)	CIM (LOD)	CIM (R^2)	PVE
CIM	**Chlorophyll (Spad)**	8	18.7 - 28.7	4.404	3.4	0.12	12.7
CIM	**Leaf mass Area**	4	76 - 84	-7.146	3.39	0.12	12.7
CIM	**Central vascular bundle**	13	38	-0.435	5.27	0.19	19
CIM	**Depth of adaxial hypodermis**	8	66.7 - 70.7	-0.051	3.55	0.13	13.3
CIM	**Width of adaxial cell**	8	70.7	-0.026	3.37	0.12	12.6
CIM	**Width of abaxial cell**	2	20 - 22	-0.005	3.01	0.11	11
CIM	**Vertical symmetry**	10	0 - 34	0.126	4.63	0.16	16.9
CIM	**Indent width mean**	8	34 - 36	-1.438	3.86	0.14	14.3
CIM	**Indent depth mean**	8	34 - 36	0.371	3.85	0.14	14.3
CIM	**Vapour pressure deficit**	13	42	0.445	3.97	0.16	16
CIM	**Number of clusters/ FV**	13	42	-140.4	5.38	0.19	19
CIM	**Width of inner vascular bundles**	2	8 – 18	-0 .048	4.03	0.14	14
MIM	**Leaf size**	9	14-20	-7498.4	4.45	0.16	16
MIM	**Leaf perimeter , area ratio**	2	22-28	-1.54	3.3	0.12	12

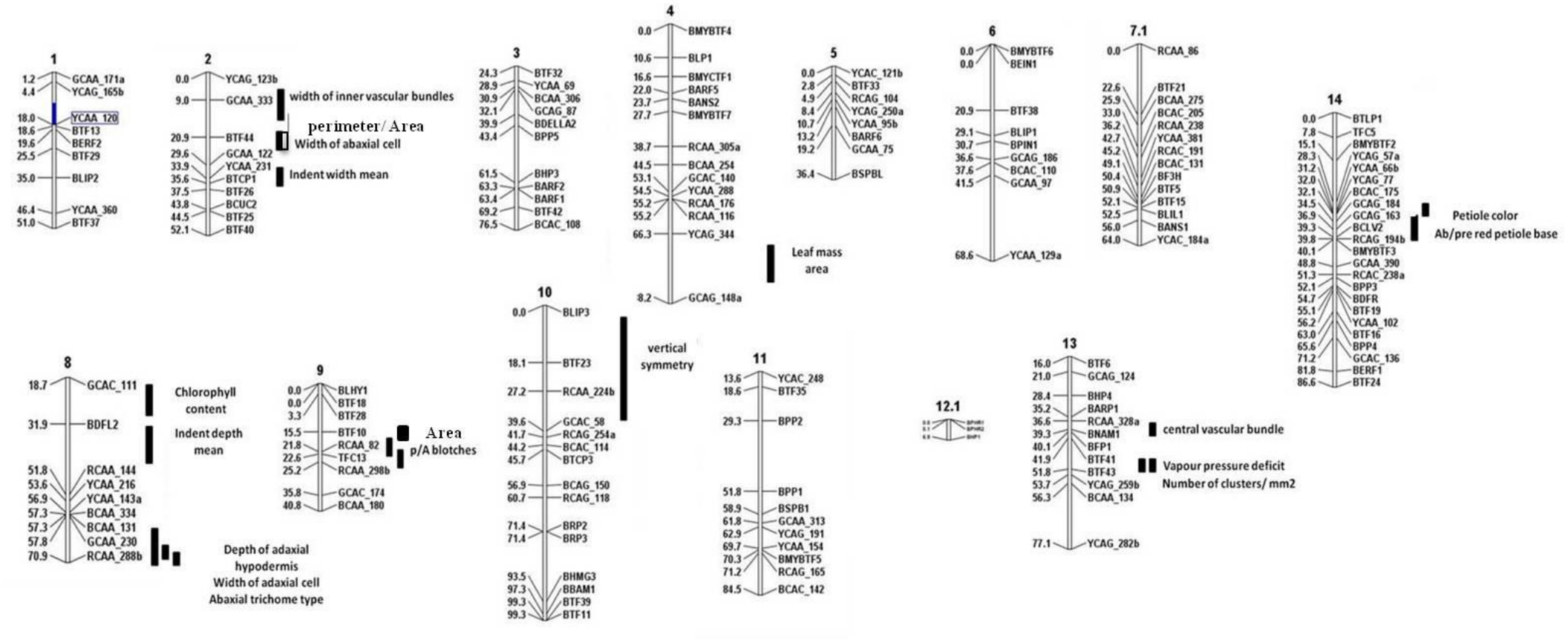

Figure 7:65: Locations of significant QTLs on the 14 chromosomes. Significance threshold for each trait was determined through resampling the data, with 500 repetitions, and chromosome walk speed was set at 2cM. Shown are the 14 linkage groups of the *Begonia* genetic map where significant QTLs localized. Genetic distances (in cM) and marker names are provided.

Table 7.2: Trait correlations between different measures of ecophysiological, leaf shape and anatomical attributes. Spearman rank order correlation analysis was used in R software to assess relationship between traits. Significance levels used are 0.05 (*).

	VpdL	ver.symm	Iwm	Idm	LMA	Spad	wdh	wdc	wbc	NscF	Ncvb	wivb	Area	SP2/A2
(VpdL)	1													
	0.09	1`												
(ver.symm)														
(Iwm)	0.16	-0.14	1											
(Idm)	0.10	-0.17	0.70	1										
(LMA)	0.44*	0.07	0.09	0.05	1									
(Spad)	0.01	0.21*	0.10	-0.07	0.11	1								
(wdh)	0.07	0.20*	0.03	-0.12	0.14	0.00	1							
(wdc)	0.38*	0.05	0.24*	0.03	0.35*	0.09	0.57*	1						
(wbc)	0.42*	0.09	0.26*	0.07	0.33*	0.12	0.37*	0.70*	1					
(NscF)	-0.24*	0.03	-0.05	-0.02	-0.11	0.00	-0.06	-0.40*	-0.29	1				
(Ncvb)	-0.23*	-0.24*	0.04	0.13	-0.15	-0.06	-0.17	-0.15	-0.24	-0.05*	1			
(wivb)	0.19*	-0.02	0.36*	0.21*	0.12	0.14	0.11	0.33*	0.49	-0.19*	0.00	1		
Area	-0.10	0.15	0.33*	0.20*	0.03	0.14	0.08	0.19*	0.28	-0.09	-0.08	0.45	1	
SP2/A2	0.06	-0.15	0.35*	0.83	0.02	-0.03	-0.12	-0.01	-0.007	-0.04	0.05	0.07	0.07	1

Table 7.3: Markers of physiological, leaf shape and anatomical traits.

Trait	Nearest Marker	Function of Arabidopsis orthologs
Chlorophyll (Spad)	GCAC_111	N/A
Leaf mass Area	GCAG-148a	N/A
Leaf Area	BTF10	DNA binding protein
Squared perimeter/ Area	BTF44 -GCAA_122	TCP family transcription factor , leaf morphogenesis
Central vascular bundle	BTF41	Encodes a family of actin bundlers , expressed in pollen
	RCAA-328a	N/A
	BNAM1	N/A
Depth of adaxial hypodermis	GCAA-230	N/A
	RCAA-288b	N/A
Width of adaxial cell	RCAA-288b	N/A
Width of abaxial cell	BTF44	TCP family transcription factor
Vertical symmetry	BL1P3	DNA binding, zinc ion binding
	BTF23	A20/AN1-like zinc finger family protein; DNA binding
	RCAA-224b	N/A
Indent width mean	BTCP1	TCP2 cell differentiation, leaf development &morphogenesis
Indent depth mean	RCAA-144	N/A
	BDFL2	Encodes an IAA-amido synthase involved in growth
Vapour pressure deficit	BTF41	Encodes a family of actin bundlers , predominantly expressed in pollen
Number of clusters/ FV	BTF41	
Width of inner vascular bundles	BTF44	TCP family transcription factor
	GCAA-333	TCP family transcription factor
Petiole colour	GCAG-163	N/A
Ab/pre Red petiole base	GCAG-163	N/A
	RCAG-194B	N/A
	BCLV2	Serine Threonine protein kinase involved in meristem regulation
	BMYBTF3	MYB DOMAIN PROTEIN 3, response to salt stress
Ab/pre trichome type	RCAA-288B	N/A
Ab/pre blotches	TCF13	IAA protein family. Involved in lateral root development.
	RCAA-82	N/A

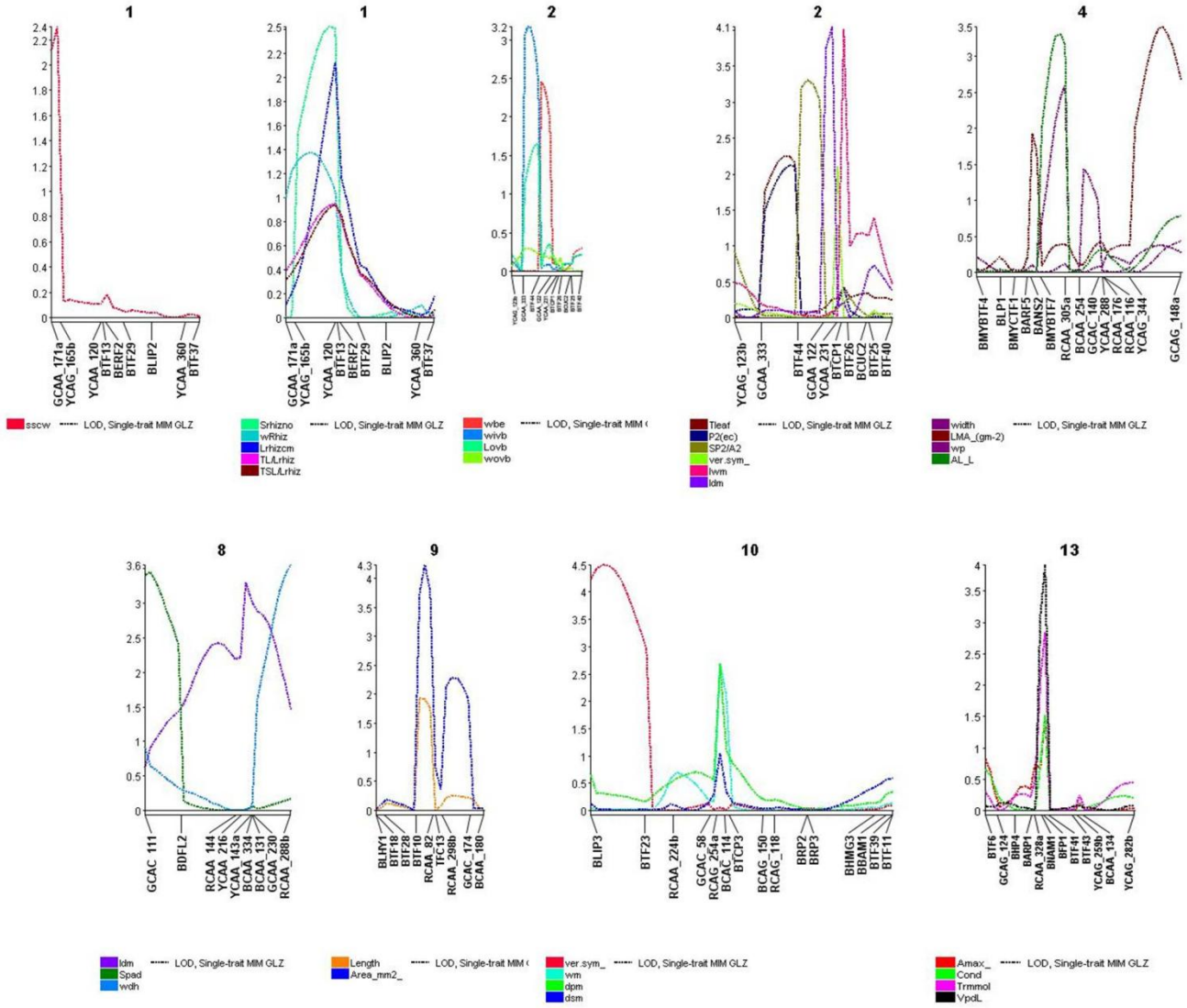

Figure 7:66: Seven linkage groups of the *Begonia* genetic map where significant QTLs and prominent peaks for traits co-localize.

7.4 Conclusions:

In this study, the aim was to identify QTLs for physiological, anatomical and morphological traits in *Begonia* section Gireoudia under green house conditions. Overall a relatively small number of QTLs were detected in this study. Out of seventy-four traits analysed QTLs were resolved for three binary, two ordinal and eleven continuous traits (Table 7.1). This suggests that there are a small number of QTLs with large effects controlling many traits, with a subset being detected in our mapping study. Having a few loci of large effect controlling the leaf traits between *B. plebeja* and *B. conchifolia* could have important evolutionary implications, as alleles at these loci could be quickly fixed by strong selection or may be lost by genetic drift in small populations (Louthan, 2011). Future fine mapping and comparisons of sequence variation at these loci in other *Begonia* species may reveal more about these traits.

No significant QTLs were resolved for net assimilation rate and stomatal conductance in this survey, yet the analysis was able to identify a region on linkage group 13 that harboured a significant QTL peak for vapour pressure deficit. Non-signficant peaks for net assimilation rate, stomatal conductance, and leaf transpiration also localized to this region. This suggests that this region might be involved in controlling traits involved in regulating net assimilation rate and stomatal conductance. The significance of differences for net assimilation rate and stomatal conductance could have been obscured by unavoidable microclimatic variability for temperature, soil water content, and VPD during growth and measurement.

Chromosome 13 also harboured significant QTLs for number of stomatal clusters per mm^2 and number of central vascular bundles. Since stomata regulate stomatal conductance and net assimilation rates in plants, a locus regulating stomatal patterning could affect all the

physiological measurements, and maybe developmentally linked with the establishment of vascularisation patterns in the expanding leaf.

Five chromosomes harboured significant QTLs for leaf size and shape related traits. Chromosome 2 harboured significant QTLs for indent depth mean and indent width mean. Both the QTLs co-localized on the same region indicating that this region is involved in controlling leaf serration. A significant QTL for indent depth mean was also resolved on chromosome 8 indicating multigenic control of leaf serration traits in *Begonia*. Significant QTLs for average leaf length, leaf size and leaf vertical symmetry were resolved on chromosome 4, 9 and 10 respectively (Figure 7.66). Several prominent peaks were also observed for leaf size and shape traits on chromosome 2 and 4 (Figure 7.66).

Two chromosomes harboured prominent QTL peaks for leaf production traits. A prominent peak QTL for number of scars and leaves produced per cm^2 of rhizome was resolved on chromosome 11. As might be expected number of leaves produced per cm^2 of rhizome also had a QTL in this region. A second region on linkage group 1 had a number of insignificant QTL peaks for rhizome number, width of rhizome, length of rhizome, total number of leaves and scars per cm length of rhizome, suggesting this region may also have a role in controlling traits related to plant architecture and leaf production, which has been somewhat obscured due to environmental sensititivy of these traits.

QTLs for traits related to internal leaf anatomy were resolved on chromosome 8 and 10. Significant QTLs were resolved for depth of adaxial hypodermis on chromosome 8. A significant QTL for depth of mesophyll was resolved on chromosome 10. A prominent peak for depth of mesophyll co-localised with the QTL for depth of spongy mesophyll. This suggests that chromosome 8 and 10 could carry important genes that affect the division,

elongation and arrangement of cells within the leaf. Chromosome 1 also harboured a prominent peak for sub stomatal cluster width. Prominent peaks for vascular bundle traits including width of abaxial epidermis, width of inner vascular bundles and length of outer vascular bundles were resolved at chromosome 2. However, the peaks were not significant.

By identifying clusters of coincident QTLs for different correlated traits, it has been shown that there is a clear genetic basis for trait correlations that are observed in the mapping population (e.g. between indent width mean and width of adaxial cell, or between vapour pressure deficit and number of clusters per mm^2). QTL clustering may indicate pleiotropy or genetic linkages. The mechanisms of pleiotropy and linked genes could further be distinguished with the help of further genetic and molecular characterisation.

In this study a few major QTLs were resolved for many leaf traits. However, the analysis failed to detect many genes of small effect. The detection of small effect QTLs could have been obscured due to environmental variability, the relatively low number of plants used in the study and the low number of markers used. A first-generation backcross population was used instead of an F_2 population in order to generate an appropriate number of progeny for QTL analysis. This could have also hindered the detection of weak QTLs for the traits in genus *Begonia* (Mauricio, 2001). QTLs were also not detected for ecologically important traits such as nitrogen content. QTL mapping studies indicate that larger numbers of QTLs are found in stressed than in unstressed environments (Teulat et al., 2000). So it is possible then that no significant QTLs were found for percentage nitrogen content because our plants were not stressed. In the future further studies on hybrids in the habitats that these species are found in might identify QTLs not found in the present survey as well as additional QTLs for the traits for which QTLs are already identified in this study.

CHAPTER 8. Discussion

Each species in section Gireoudia has a unique geographic distribution pattern corresponding to a unique combination of environmental variables. The habitats are remarkably variable with respect to precipitation in particular (Table Appendix 1) (Nikki Harrison, unpublished). The variation in habitats may be related to variations in leaf structure and function. However, it is very difficult to define accurate habitat envelopes for tropical understory plants as microclimate is very variable in these environments (Rascher et al., 2012, Lima and Gandolfi 2009). A full analysis of the correlations between habitat and structure/function would require many site visits and direct ecological measurements.

Based on the observed variation, *Begonia* section Gireoudia forms a complex and unique group that stands out from other taxa at global scale on the basis of leaf function and resource use strategy traits as well as in leaf anatomy (Figure 8.1). Traits directly related to leaf function such as photosynthesis and stomatal conductance values are very low. A_{max} ranged from 0.33 µmol m^{-2} s^{-1} to 5.40 µmol m^{-2} s^{-1} and stomatal conductance ranged values from 13 mmol m^{-2} s^{-1} to 106 mmol m^{-2} s^{-1}. Such low photosynthetic and stomatal conductance values could be related to low metabolic activity in habitats limited for light.

Most of the rhizomatous species in *Begonia* section Gireoudia occur in deep shade on tree trunks and rocks. A review of maximum rates of net photosynthesis of such epiphytic and saxicolous species yielded mostly low values (average: 2.6 µmol m^{-2} s^{-1}) (Larcher, 2003). A recent paper stresses the importance for epiphytes of quick response to fluctuating light conditions (Rascher et al., 2012), so a key photosynthetic adaptation may not be to

continuous low light but to allow plants to make the most productive use of sunflecks. Further analysis of the species' photosynthetic activity under a range of conditions is needed to determine whether any of section Gireoudia show enhanced response to changes in light conditions.

Since Crassulacean acid metabolism (CAM) is very common among tropical forest plants, there is a possibility that *Begonias* have evolved CAM. Water has been suggested to be a limiting factor for epiphytes (Zotz, 2001), and Crassulacean acid metabolism serves as water saving pathway in the epiphytic habitat (Reyes-García et al., 2008). *Begonia*s of section Gireoudia are certainly more robust to drying out than others of the genus, this may account for their popularity as house plants (Tebbit, 2005). The values observed for species in *Begonia* section Gireoudia are also well within the range of CAM plants. However in a recent CAM study conducted on *Begonia* species by Emily Johnston (unpublished), no significant increase in total acid or malic acid content was determined between dawn and dusk foliage samples from the sampled species as one would expect for CAM plants. The particularly high acidity (pH2) of the *Begonia* leaves possibly due to high oxalic acid content could have masked malate fluctuations in the study and needs further investigation (Rose and Hurd-Karrer 1927). Another important finding in Emily's study was the high carbon isotope ratio of *B. ampla*, (δ13C (‰) -23.78) suggestive of the fact that this species could use CAM to an extent. Other features of section Gireoudia such as leaf succulence and drought deciduousness also are associated with Crassulacean acid metabolism in some groups (Ng and Hew, 2000; Benzing et al., 1983; Benzing, 1990).

In the light of the above facts, it can be concluded that CAM in section Gireoudia is certainly a possibility and worth further investigation. Further work should also focus on other

mechanisms by which these plants may be dealing with water stress as epiphytes or in seasonally dry environments.

Values for traits such as LMA and LDMC (indicative of resource use) are very low in *Begonias* when compared with the values observed globally. The LMA values ranged from 49 g m^{-2} to 195 g m^{-2} and LDMC values ranged from 21g m^{-2} to 224.9 g m^{-2}. Such low leaf mass areas and leaf dry matter content values along with low net assimilation rate makes *Begonia* a unique group that stands out from the rest of the taxa (Figure 8.1).

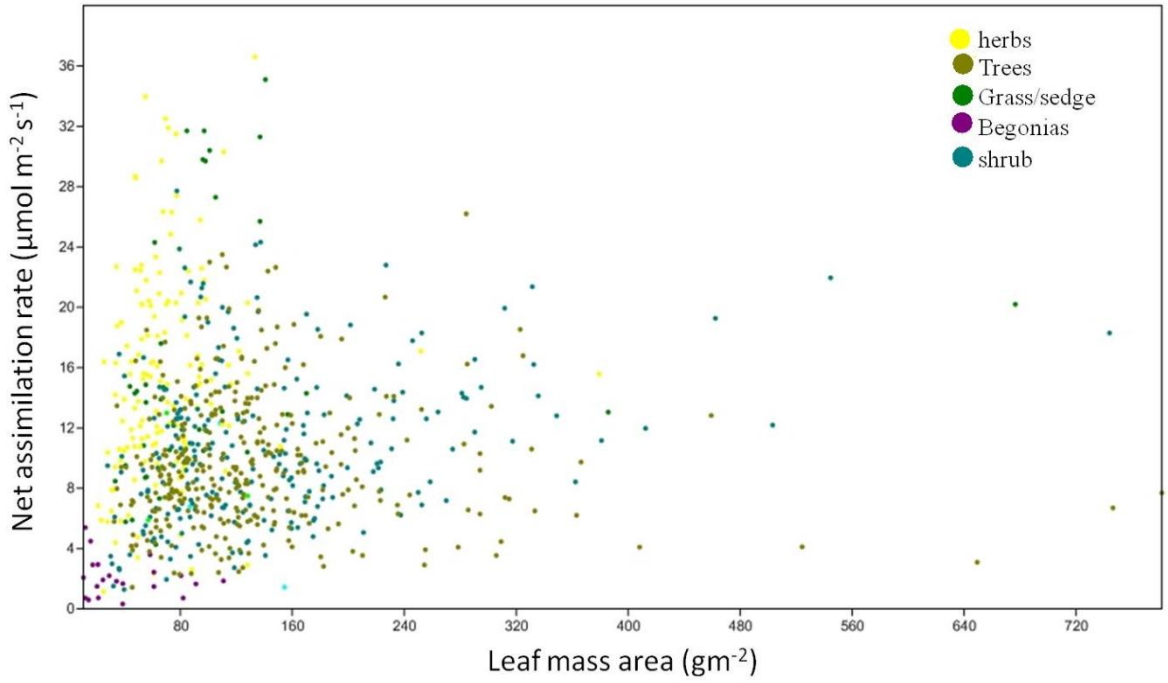

Figure 8.1: Two way trait relationships between leaf mass area (g m^{-2}) and net assimilation rate (umol m^{-2} s^{-1}). The graph is plotted using data from Wright et al., 2004 with data from this study added.

One possible explanation of such low LMA could be the plants' low daily photon irradiance (DPI) (Garnier and Freijsen, 1994). The low light intensities would restrict the plants' ability to invest large amounts of energy in the leaf. The leaf dry matter content values are also very low which could be explained by the presence of the multiple water filled hypodermis. Only a

small proportion of the leaf consists of the mesophyll and much of the volume of the leaf is the water filled hypodermis (Solereder and Meyer, 1929).

Relative water content is a useful indicator of plant water balance, since it expresses the relative amount of water present in the plant tissues. The relative water content (RWC) of a plant tissue is expressed by RWC (%) = [(FM - DM)/(TM - DM)] * 100, where, FM, DM, and TM are the fresh, dry and turgid masses, respectively, of the tissue. Relative water content was measured in this project but there were problems which rendered the measurements unreliable. During the rehydration process, it appeared that the sampled leaves were losing water instead of gaining it. This could be due to leaves undergoing a large osmotic adjustment, possible as *Begonia* leaves manage to maintain pHs as low as pH2 in some cells (Johnson unpublished data, Rose and Hurd-Karrer 1927).

Water potential measures the energetic status of water inside the leaf cells (Slatyer and Taylor, 1960). Water potential of leaves is usually measured with a pressure bomb. Mechanical problems with the pressure bomb available to me prevented me from further exploring the water potential for *Begonias*. The measurement of these two traits would have certainly have provided valuable information on the physiology of these species.

Trait- trait correlations across the species in section Gireoudia revealed interesting patterns. Some of these traits are correlated with each other in apparently straightforward biological relationships e.g, the variation among *Begonia* species in stomatal conductance and positive correlations of this character with net assimilation rate observed in the current study appear to reflect the gas exchange limitation of photosynthesis ($r^2 = 0.48$, $P < 0{\cdot}05$). Others such as the linkage of high A_{max} with high N_{mass} as well as high A_{max} with low LMA were not found across species in section Gireoudia. The absence of the correlation patterns observed

globally among ecological leaf traits could be attributed to the small data set used here as well as the small range of variation observed for the traits.

A detailed analysis of variation between *B. plebeja* and *B. conchifolia* detected significant differences between the species that are consistent with generalised adaptive patterns typical of sun and shade plants. Moreover, significant differences were found for key leaf economics traits such as nitrogen and leaf mass area between the species. *B. plebeja* has characteristically higher nitrogen content and low leaf mass area and vice versa. The high nitrogen content of *B. plebeja* could be linked to the slightly higher net assimilation rates via greater investment in photosynthetic enzymes.

The low leaf mass area suggests that *B. plebeja* invests a low amount of its resources in leaves. *B. plebeja* have leaves that are large and thin and require less force to tear apart or puncture and have a short life-span, deciduous in some populations. *B. conchifolia* leaves have low nitrogen content and high leaf mass area. The high leaf mass area could be linked with the long leaf life which requires robustness. The lower nitrogen content could be linked to lower net assimilation rates and may impart lower palatability to the long lived *B. conchifolia* leaves.

The two closely related species do span a large amount of the variation seen in the section as a whole and typical of two distinct environments. I used a mapping population to try and genetically characterize this variation in order to determine if the switch between environments was a genetically easy change to make.

The mapping population B08-360 was transgressive for most of the traits. Interspecific transgressive segregation for ecological tolerances such as high temperatures in tropical

deciduous forests could have played an important role in the success of hybrids in *Begonia* section Gireoudia as it appears to have done in other genera such as *Helianthus* (Rieseberg, 1997). Speciaton rates are high in section Gireoudia (Nicola Harrison, PhD thesis 2012) and hybridisation in this section has been observed in the wild (McMillan et al., 2006, Twyford PhD thesis 2012). However, so far, introgression and hybrid speciation have not been observed (Twyford PhD thesis 2012). Hybridisation may have been an important factor in the colonisation of the new environments of North America after the rise of the panama isthmus.

Segregation analysis revealed that traits such as colour of stipules and margin colour strength are under simple genetic control. Some of the trait variation that is qualitative in nature and most of the quantitative trait variation in the mapping population seems to have complex genetic inheritance patterns. This study also identified QTLs for physiological and anatomical traits in *Begonia plebeja* and *B. conchifolia* under green house conditions. The analysis resolved QTLs for three binary, two ordinal and fifteen continuous traits. The genetic architecture of continuous leaf traits in genus *Begonia* appears to be largely influenced by a few loci of large effect. This suggests that there are a small number of QTLs with large effects controlling these traits, with a subset being detected in our mapping study. The study also identified clusters of coincident QTLs suggesting a clear genetic basis for trait correlations observed in the mapping population. Localization of QTLs for multiple traits to the same chromosomal region has also provided preliminary evidence of regulation by the same set of genes. Some of the traits also seen to be linked in the Gireoudia-wide survey, e.g, net assimilation rate and stomatal conductance. This suggests recombination to link adaptive suites of traits, or, in cases such as stomatal conductance and Amax, that the traits are strongly functionally linked.

Several QTLs were also identified near sequenced markers though in no cases was an obvious candidate gene for the trait identified. The next step is to locate further candidate genes linked to these QTLs by improving the scaffolding of the draft genome. For future progress, methods such as association mapping could become an important tool for testing genes identified as candidates on the basis of function and map location.

For future studies QTL mapping in *Begonias* should be carried out with a sample size of at least several hundred individuals to reliably detect the QTLs that were not resolved in this study. Replication of individually by cuttings would help remove some of the environmental variation which may be obscuring QTLs for eco-physiological traits (Beavis, 1994). It would also be very valuable to measure the traits in common gardens in the parental species habitats. QTL mapping studies indicate that larger numbers of QTLs are found in stressed than in unstressed environments (Teulat et al., 2002). It would be very exciting to repeat the analysis using different parental species (but also sun/shade pairs) to see if the same QTLs are identified, suggesting a limited number of evolutionary pathways to a set phenotype.

At a broad scale this study is a little contribution to the important and unanswered questions relating to the explanation of high tropical species diversity. Mittelbach et al. (2007) recently reviewed that the three primary categories of explanations for tropical biodiversity including historical, evolutionary and ecological hypotheses. The work carried out in this thesis on species rich tropical genus *Begonia* adds a genetic perspective, suggesting that mutations in few major loci could lead to habitat divergence among populations and species in the tropics.

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Appendix 1.2: Habitat data for some of the Gireoudia's.

	dd_long	dd_lat	ALT_EX	TMIN	TMAX	PREC
B. lindleyana	-87.19	13.82	571.43	18.14	28.97	181.61
B. peltata	-94.50	17.63	1045.88	14.97	26.38	139.69
B. conchifolia	-83.58	9.87	1138.83	15.79	25.28	236.43
B. cardiocarpa	-85.58	12.11	815.05	16.98	26.98	163.20
B. carolinefolia	-96.20	18.58	894.50	15.61	26.86	201.14
B. corredorana	-83.60	9.19	499.02	18.72	29.38	287.86
B. heracleifolia	-95.10	18.10	540.15	17.58	28.52	191.83
B. hydrocotilifolia	-96.91	19.29	1147.29	13.70	25.56	106.00
B. involucrata	-84.08	9.82	1713.69	12.39	21.80	232.38
B. multistaminae	-96.59	19.30	1188.44	13.94	24.16	177.04
B. nelumbifolia	-92.98	17.61	499.48	18.00	28.36	203.20
B. plebeja	-85.71	11.62	662.89	18.23	28.55	168.69
B. sericoneura	-85.51	13.07	449.45	19.07	28.75	204.88
B. squarrosa	-96.47	16.18	1324.40	13.52	27.50	109.55
B. stigmosa	-100.19	18.72	1447.50	13.65	26.64	123.37
B. thiemie	-92.98	17.62	257.30	19.72	29.72	221.59
B. multinervia	-83.87	9.67	496.70	18.88	29.04	266.96

Appendix 6.2: Code for generation of heat map

```
setwd("/home/tobi/Desktop/Tobi meeting/R_sheets")

source("utils.R") # to load vec.to.mat

rawdata <- read.table("SpearmanResults.csv", sep = ",", header = TRUE,
                      row.names = 1, stringsAsFactors = FALSE)

# construct vector containing only significance
values <- rawdata[, "significance"]
names.vec <- paste(rawdata[, "character1"], rawdata[, "character2"], sep = "-")

names(values) <- names.vec
mat.pvals <- vec.to.mat(values, sep = "-")

#test <- as.dist(t(mat.pvals))


heatmap(t(mat.pvals), Rowv = NA, Colv = NA, symm = TRUE, revC = TRUE)
```

Appendix 7.3: Chi square R steps for resolving categorical QTLs

```
setwd("/home/tobi/Desktop/Tobi meeting/R_sheets")

matA <- read.table("genotypes.csv", sep = ",", row.names = 1)
matB <- read.table("morphology.csv", sep = ",", row.names = 1)

#source("pairwise.chisq.R")
source("pairwise.fisher.R")
source("utils.R")

#pairchi <- pairwise.chisq(matA, matB)
pairfisher <- pairwise.fisher(matA, matB)

# parameters for adjusting p-values

mat_vector <- mat.to.vec(pairfisher) # convert matrix to vector for p.adjust
pvals_adj <- p.adjust(mat_vector,  method = "BH")
```

```
pvals_adj <- vec.to.mat(pvals_adj) # transform p-values back to matrix

sig.p <- which(pvals_adj <= 0.05, arr.ind = TRUE)

sig.correl <- matrix(nrow = nrow(sig.p), ncol = 3)
colnames(sig.correl) <- c("var.1", "var.2", "p")
for (i in 1:nrow(sig.p)) {
  sig.correl[i, 1] <- rownames(pvals_adj)[sig.p[i, 1]]
  sig.correl[i, 2] <- colnames(pvals_adj)[sig.p[i, 2]]
  sig.correl[i, 3] <- pvals_adj[sig.p[i, 1], sig.p[i, 2]]
}
```