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Environmental and genetic influences on growth characteristics in a full sib family of *Populus nigra*

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Abstract

Growth related traits in trees are fundamental components of survival and productivity in natural and planted forests. This wide category of traits refers to many attributes, such as aboveground biomass production and spring bud phenology. Wood trees provide a sustainable source of carbon-neutral bioenergy, and plant phenology represents a serious ecological and evolutionary trade-off between survival and growth. Aboveground biomass production and allocation are the result by interaction among climate condition, genetics variance and silvicultural management. Phenology is responsive to global warming, especially spring bud flush that is predominantly controlled by temperature. The aim of this study was to improve the knowledge of genetic basis and environmental influence related to: (i) biometric traits and biomass production; (ii) bud flush dynamics focusing on the influence of temperature on this process. A *Populus nigra* full-sib family (POP5) originating from a controlled cross between parental genotypes from contrasting climate was studied at different locations and in different growing seasons.

Different linear regression models, based on biometric traits, were developed to estimate biomass yield for each environment. Model accuracy was very high and has been possible to standardize estimation models between growing seasons in the same location. High phenotypic variance was observed in the trials, in particular among plantations with different age root system. Northern parent "58-861" shown greater adaptability and productivity (11.6 t ha^{-1} $year^{-1}$) compared to the southern parent "Poli" (3.4 t ha^{-1} $year^{-1}$). Mean POP5 productivity was 11.3 t ha^{-1} $year^{-1}$. Very low values of heritability (H^2<0.25) were observed for the different biometric and biomass traits in the trials. The relative importance of environment, genotype and genotype × environment (G×E) interaction effects on total phenotypic variation of growth traits was estimated. Significant G×E interaction was observed for sites characterized by lack of coppicing at the end of first growing season. A large number of QTLs, 41 on paternal map and 44 on maternal map were found, corresponding to 11 and 8 genomic regions, respectively. QTLs were characterized by small or modest effect (average PVE=6%). Four common molecular markers (ORPM_127, PMGC_2839, PMGC_2423, PMGC_520) were found between maternal and paternal maps on four different linkage groups (LG IV, V, VI, XV). On LG XIX, five QTLs were found for height using multi-environment analyses.

A protocol was developed for black poplar (*P. nigra*) to dissect the bud burst spring phenology in different stages, from the swelling of the buds to the complete leaf formation, passing through three intermediate phases. Cumulative average temperature above 10°C (CAT_{10}) and cumulative minimum temperature below 5°C (CMT_5) were used to describe phenotypic and genetic variance of the bud flush stages. Two growing seasons were compared in the site of Viterbo. Both growing seasons showed similar CAT_{10} values for the initial stage of bud flush, despite seven days of difference for the bud flush initiation observed between the years. Southern parent was more sensible to temperature variation than the northern parent. Greater fluctuations in temperatures increased the extent of phenotypic variance among genotypes during the process than stable temperatures. Moderate to low values of heritability (H^2<0.50) were observed for the different stages in the two years. No significant G×E interaction was observed. A large number of QTLs, 28 on paternal map and 26 on maternal map, were found, corresponding to 9 genomic regions for each parent. QTLs were characterized by small or modest effect (average PVE=6.7%), without common genomic regions between parental maps.

These results highlight the complex nature of the traits involved in woody biomass determination and during bud flush and leaf elongation process.

Key-words: *Populus nigra*, aboveground biomass production, bud flush, G×E interaction, QTLs.

Riassunto

I caratteri legati alla crescita delle piante sono componenti fondamentali della sopravvivenza e della produttività in foreste naturali e piantagioni. Tra questi caratteri rientrano la produzione di biomassa epigea e la fenologia primaverile delle gemme. Gli alberi forniscono una fonte di energia rinnovabile a bilancio zero di carbonio, e la fenologia rappresenta un importante compromesso ecologico ed evolutivo tra sopravvivenza e crescita. La quantità di biomassa epigea prodotta e la sua allocazione sono il risultato dell'interazione tra condizioni climatiche, variabilità genetica e pratiche selvicolturali. La fenologia è influenzabile dal riscaldamento globale, in particolar modo quella primaverile, il "bud flush", che è principalmente controllato dalla temperatura. L'obiettivo di questo studio è stato migliorare la conoscenza delle basi genetiche e dell'influenza ambientale relativamente a: (i) caratteri biometrici e produzione di biomassa; (ii) dinamica del bud flush, focalizzandosi sull'influenza delle temperature su questo processo. Una famiglia full-sib di *Populus nigra* (POP5), originata da un incrocio controllato tra parentali di diversa provenienza, è stata studiata in differenti località e stagioni di crescita.

Diversi modelli di regressione lineare, basati su caratteri biometrici, sono stati sviluppati per stimare la biomassa prodotta in ogni ambiente. I modelli sono risultati essere molto accurati ed è stato possibile standardizzare le equazioni di stima tra diverse stagioni di crescita nello stesso sito. Una elevata variabilità fenotipica è stata osservata tra le sperimentazioni, in particolare tra piantagioni con diversa età dell'apparato radicale. Il parentale settentrionale "58-861" ha mostrato maggior adattabilità e produttività (11.6 t ha^{-1} $anno^{-1}$) del parentale meridionale "Poli" (3.4 t ha^{-1} $anno^{-1}$). La produttività media di POP5 è stata di 11.3 t ha^{-1} $anno^{-1}$. Valori molto bassi di ereditabilità (H^2<0.25) sono stati osservati per i diversi caratteri biometrici e di biomassa nelle sperimentazioni. È stata stimata l'importanza relativa degli effetti dell'ambiente, del genotipo e dell'interazione tra genotipo ed ambiente (G×E), rispetto alla variabilità fenotipica totale. Una significativa interazione G×E è stata osservata per i siti caratterizzati dalla mancanza di ceduazione al termine della prima stagione di crescita. Un elevato numero di QTLs (41 e 44, rispettivamente sulla mappa paterna e materna) sono stati trovati, concentrati in 11 e 8 regioni genomiche. I QTL hanno mostrato un effetto basso o modesto (in media PVE=6%). Quattro marcatori molecolari (ORPM_127, PMGC_2839, PMGC_2423, PMGC_520) sono stati trovati in comune tra le mappa materna e paterna, su quattro differenti gruppi linkage (LG IV, V, VI, XV). Sul LG XIX, sono stati trovati cinque QTL per l'altezza, ottenuti da ogni analisi multi - ambiente.

Un protocollo di analisi è stato sviluppato per pioppo nero (*P. nigra*) al fine di dissezionare la fenologia di entrata in vegetazione primaverile della gemma in diverse fasi, dal rigonfiamento delle gemme sino alla completa formazione delle foglie, passando attraverso tre fasi intermedie. La cumulata della temperatura media al di sopra dei 10°C (CAT_{10}) e la cumulata della temperatura minima al di sotto dei 5°C (CMT_5) sono state utilizzate per descrivere la variabilità fenotipica e genetica delle fasi. Nello stesso sito, due stagioni di crescita sono state raffrontate. Entrambe le stagioni di crescita hanno mostrato valori simili di CAT_{10} per la fase iniziale del bud flush, nonostante sette giorni di differenza di entrata in vegetazione osservati tra i due anni. Rispetto al parentale settentrionale, quello meridionale è stato più sensibile alle variazioni di temperatura. L'entità della variabilità fenotipica tra genotipi, durante il processo, è risultata essere superiore in condizioni di marcate oscillazioni di temperatura piuttosto che di stabilità di quest'ultima. Nei due anni, per le diverse fasi sono stati osservati valori di ereditabilità da moderata a bassa (H^2<0.50). Non è stata osservata nessuna interazione G×E significativa. Sono stati trovati un elevato numero di QTLs (28 e 26, rispettivamente sulla mappa paterna e materna), concentrati in 9 regioni genomiche per ogni parentale. I QTL hanno mostrato un effetto basso o modesto (in media PVE=6.7%), senza regioni genomiche in comune tra le mappe dei parentali.

Questi risultati evidenziano la natura complessa dei caratteri coinvolti nella determinazione della biomassa legnosa e durante il processo di apertura della gemma e formazione della foglia.

Parole chiave: *Populus nigra*, produzione di biomassa epigea, bud flush, interazione G×E, QTLs.

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INTRODUCTION

Poplars are considered the fastest growing trees under temperate zones and are broadly used to produce a variety of wood-based products including paper, plywood, matches, light packaging materials or wood for bioenergy. Among forest trees, they are important components of riparian ecosystems and are now accepted by the scientific community as a woody model tree which is necessary needed to study the unique processes that occur in perennial plants, such as secondary wood formation, bud set, bud flush and dormancy (Jansson and Douglas, 2007). Poplars offer several advantages as a model system, including rapid juvenile growth (Bradshaw et al., 2000 ; Dickmann, 2001), high levels of natural variations in wild populations (Jansson and Douglas, 2007), easy of sexual and asexual propagation (Stettler and Bradshaw, 1996), small genomic size (about 550 million base pairs, 4× larger than *Arabidopsis* and one fortieth the size of the *Pinus* genome) (Tuskan et al., 2006), facile transgenesis, plant transformation and regeneration capabilities (Wullschleger et al., 2002). *P. nigra* is one of the most important parents to produce Euramerican hybrids in breeding programs to increase wood production and to be used in short rotation intensive culture.

Biomass production and spring bud phenology are growth related traits, which are fundamental components of tree survival and productivity in natural and planted forests.

From an utilitarian perspective, growth (i.e. wood dry weight and wood volume increment) in trees is synonymous to productivity. It represents the foremost target trait for intensive woody biomass production in any tree breeding program, whether the final application is structural wood, engineered wood products, pulp and paper, or energy. Sustainability of any forest-based industry depends on a constant supply of wood, optimal land use, and the efficiency of scale in a capital-intensive operation (Grattapaglia et al., 2009)

Given the influence of temperature on the phenology of trees, the break of bud dormancy and the leaf formation may be affected by globally climatic warming. It can influence the frequency of late frost damage, the survival and, eventually, the distribution of forest trees, caused by less closely adaptation to the local altered environment (Hänninen and Tanino, 2011). The *Populus nigra* riparian forests have been largely lost due to the spread of agriculture and other human activities. The genetic diversity, that is considered to be important for long-term survival of natural populations and for the ability to adapt to changing environmental conditions (Grattapaglia et al., 2009), is seriously threatened for this species (Lefèvre et al., 1998). In the light of these considerations, the main objective of this work is to contribute to the knowledge of the genetic and environmental control of biomass production and bud flush phenology in *P. nigra*.

1. STATE OF THE ART

1.1. Populus nigra L.

1.1.1. Taxonomy and distribution

Populus nigra L. (black poplar) is a pioneer angiosperm of the genus *Populus* L., which belongs to the section *Aigeiros*. *Populus* genus, together *Salix* L., is the most representative genus of the family of *Salicaceae* (Fig. 1.1). Section *Aigeiros* includes some of the major riparian poplars in the boreal hemisphere, which, along with willows, are a defining feature of these habitats. As well as black poplar, *Populus deltoids* Marsh. (eastern cottonwood) and *Populus fremontii* S. Watson (Fremont cottonwood) are included in this section. However, the placement of *P. nigra* within this section remains controversial, saw marked morphological differences between black poplar and cottonwood, and genetic affinity observed with *Tacamahaca* and *Populus* sections (Hamzeh and Dayanandan, 2004). A possible exclusion of cottonwoods, leaving *P. nigra* the sole member of section *Aigeiros*, has been proposed (Eckenwalder 1996). Generally, the extensive inter-specific hybridization and the high levels of morphological variation among poplars have posed great difficulties in species delimitation for systematic and comparative evolutionary studies (Fig. 1.1).

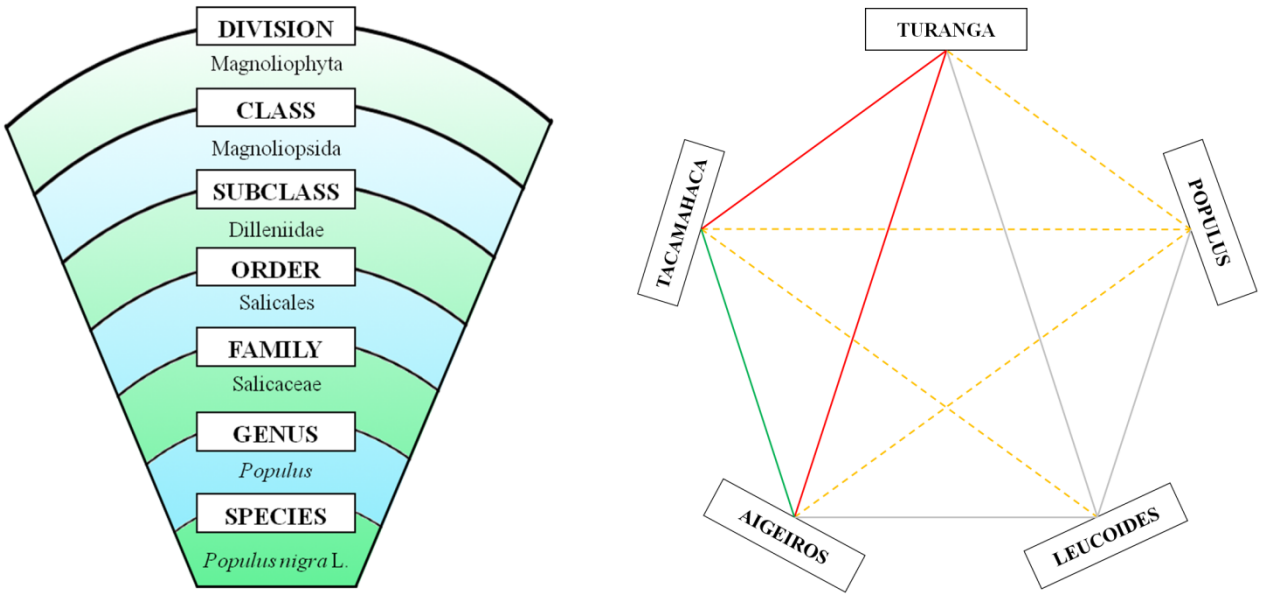

Figure 1.1: Taxonomy hierarchy of Populus nigra (on the left) and scheme of possible hybridizations among sections of genus (on the right) (modified from Zsuffa, 1975). Are shown fertile crosses (green line), difficult crosses (yellow dotted line), incompatible crosses (red line) and unknown relationships (grey line) among different sections.

Black poplar has a wide natural distribution area that extends from Atlantic Ocean (western coast of Europe, Ireland and the British Isles) to Kazakhstan and China in the east. It is present like spontaneous species all over Europe, from the Mediterranean in the south to approximately 64° latitude in the north (Fennoscandia excluded). In western Asia, it is present form Pakistan (30° N) to Severnaya river in Russia (64° N). Also, it is present in northern coast of Mediterranean Africa (Fig. 1.2).

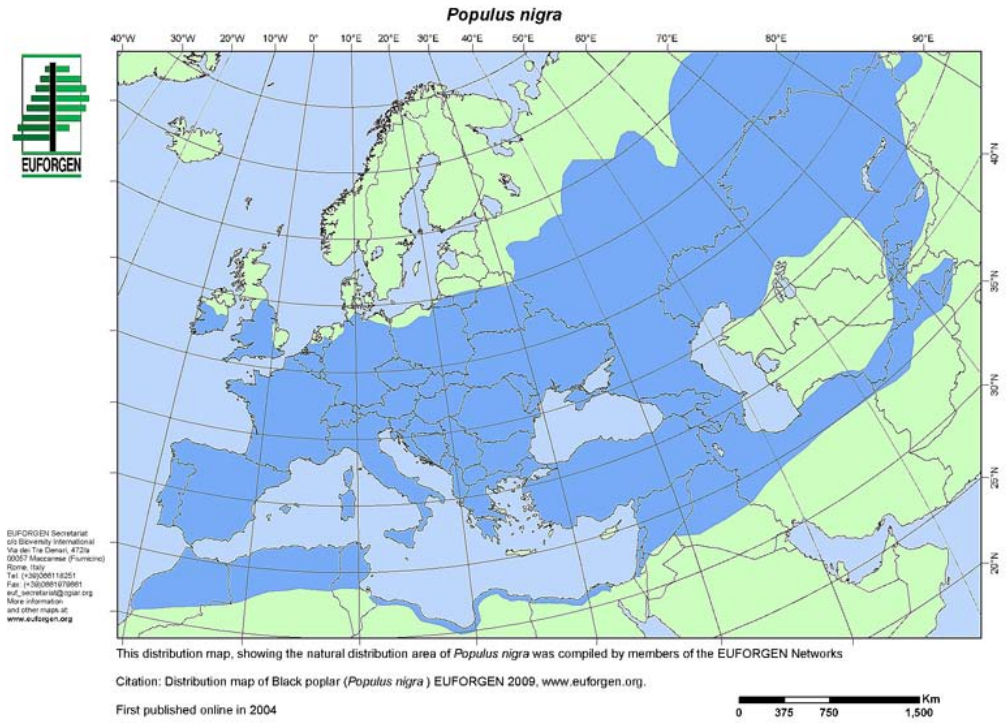

Figure 1.2: Distribution map of black poplar (*Populus nigra* L.), EUFORGEN 2009.
URL: http://www.euforgen.org/distribution_maps.html

In Italy the black poplar grows in all the national territory, between the sea level up to 1.200 m a.s.l. in the Alps , up to 1.660 m a.s.l. in the Appenines system (Vietto, 2009), that corresponds to cold and medium *Lauretum* and warm and cold *Castanetum* phytoclimatic zones.

1.1.2. Biology and ecology

Populus nigra is a rapid growth tree that reach heights of 30 m, rarely 40 m, and diameters over 2 m at maturity; generally poplars have a short lifespan (at mean 100 years), but individual of 300 years were observed (Weisgerber, 1999). The crown is rounded and wide (Fig. 1.3). *Populus nigra*, produce both prolectic and sylleptic branches. Proleptic branches grow from buds that have over-wintered in a dormant condition, while sylleptic branches elongate from new buds produced during

the current growing season. The bark is yellow-white in juvenile age and dark grey fissured at maturity. Accentuated dimorphism exists between pre-formed and neo-formed leaves of *Populus nigra* L.; pre-formed leaves are small (15-95 cm^2), dark green, rhombic in shape with a slightly lighter under-surface, whereas neo-formed leaves are larger, broader than long, and oval in shape (Dickmann and Kuzovkina, 2008). The lower angle of leaf is rounded, while the upper angle is pointed. Leaves are simple and the blade is smooth on both sides with finely toothed margins and long, flattened petioles. Black poplar is a dioecious tree, obligatory out-crossing species with female and male flowers on separate uni-sexual individuals.

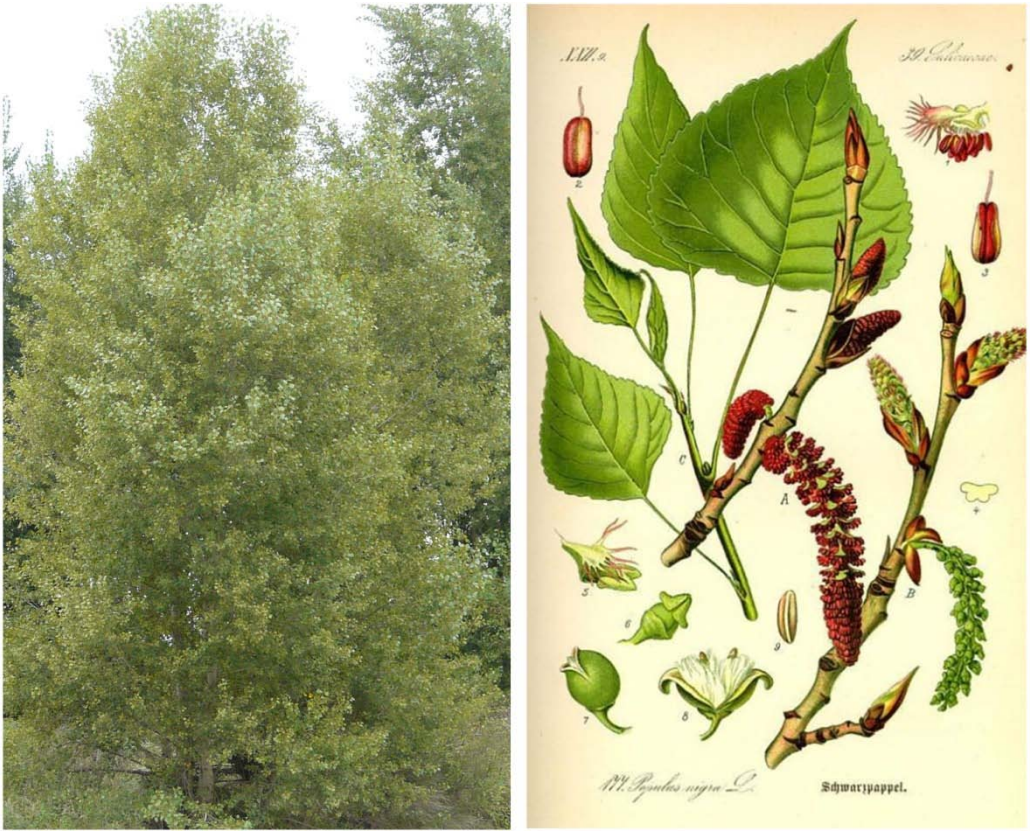

Figure 1.3: Black poplar in a natural riparian ecosystem (on the left). *P. nigra* leaves, catkins, and seeds (on the right - realized by Otto, 1885. URL: http://caliban.mpiz-koeln.mpg.de/thome/band2/tafel_020.html)

The flowers of both genders are pendulous racemes (catkins, aments), length up to 10 cm and of color green on females, smaller reddish-purple on males, located in the upper side of the foliage (Barsoum, 2001) (Fig. 1.3). Occasionally inflorescences could be hermaphroditic, producing both male and female flowers, or individual trees or clones will bear both male and female catkins (Wyckoff and Zasada 2007). Commonly males begin flowering earlier than females, so pollen is in the air when the females are receptive (Farmer and Pitcher, 1981). The preformed inflorescences are contained in specialized buds that flush in early spring (March - April), weeks prior to leaf emergence. Wind-dispersed pollen fertilizes ovule within 24 hours of arrival upon the receptive

stigma and subsequent ovule ripening, seed maturation and dispersal occurring within 3-6 weeks following fertilization**.** Mature catkins are made up of many capsules, approximately 20-50, each developed from an individual flower, with about 4–5 small seeds (2 mm) per capsule with little or no endosperm (Braatne et al., 1996). Seeds are light and cottony, so they can travel for more 10 km of distances using the wind (Wyckoff and Zasada 2007), and a secondary transport by moving water can extend this range. A remarkable quantities of seed are generally produced after 20 years old (Stanton and Villar,1996), though *P. nigra* reaches reproductive age at 10-15 years old. As for other taxa of section, black poplar shows marked periodicity in seed production, however complete reproductive failures are rare (Dickmann and Kuzovkina, 2008). The germination of seeds is generally good, but seed life is short (less than 2 weeks), and after the hydration viability will be lost in 2-3 days (Braatne et al., 1996).

Black poplar is a typical heliophilous pioneer species that colonizes river floodplains, wastelands, and other exposed sites (Fig. 1.4).

Figure 1.4: Riparian forest with black poplar along Paglia river (Acquapendente, VT - Italy.

Its prodigious production of wind-blown seed, as well as rapid growth rate, greatly facilitates this colonizing ability. *P. nigra* is a no-zonal species not linked to particular climatic area but only to the soil moisture, in fact after seasonal flooding of rivers, the moist sandy soil represents the best condition for seeds germination. It is a signature species of riparian ecosystems, and often creates plant associations with white poplar (*Populus alba* L.), field elm (*Ulmus minor* Mill.), black alder

(*Alnus glutinosa* L.), *Salix* genus and less frequently with European ash (*Fraxinus excelsior* L.) and e hornbeam (*Carpinus betulus* L.) (Gellini and Grossoni, 1997). Despite the feeling with riparian areas, black poplar doesn't tolerate anoxic conditions for long time periods. Black poplar, as all the other taxa of the *Salicaceae*, have the ability to reproduce vegetatively new individuals by vigorous root collar sprouts, stool shoots, root suckers and through arboricultural cares, with stem and root cuttings.

1.1.3. Ecological importance of Populus nigra *L.*

Black poplar typically grows on the border of alluvial, riparian, and wetland habitats and they occur in mixed forests with other hardwood trees. The natural ecological role of black poplar is the colonization of riparian zones disturbed by seasonal flooding. *P. nigra* together *Salix alba* and *Salix purpurea*, plays a key role in the morphological development of the riparian zone of large gravel rivers. The accretion of bars and islands depends on these species for their capacity to catch sand and hold the developing sediment zones (Hughes et al. 2001; Van Looy et al., 2005). Natural riparian stands decrease flood damage, they control erosion of river bank, and thanks to vegetative propagation, low cost plant material are available for bioengineering restoration. Moreover, riparian forests provide improved habitats for other organisms, with subsequent increment of ecosystem biodiversity. Poplar natural stands cover an area of 131400 hectares in Europe; they are mainly concentrated in Hungary, Spain, Romania and France (Coaloa and Nervo 2011).

Poplars are also used in phytoremediation, consisting to clean up pollutants in contaminated soils using tree plantations, which accumulate in their tissues (Chaney, 1983). A more articulate definition of phytoremediation is: “a technology that utilizes plants and then the associated rhizosphere microorganisms to remove, transform, or contain toxic chemicals located in soils, sediments, ground water, surface water, and even the atmosphere” (Susarla et al., 2002) (Fig. 1.5).

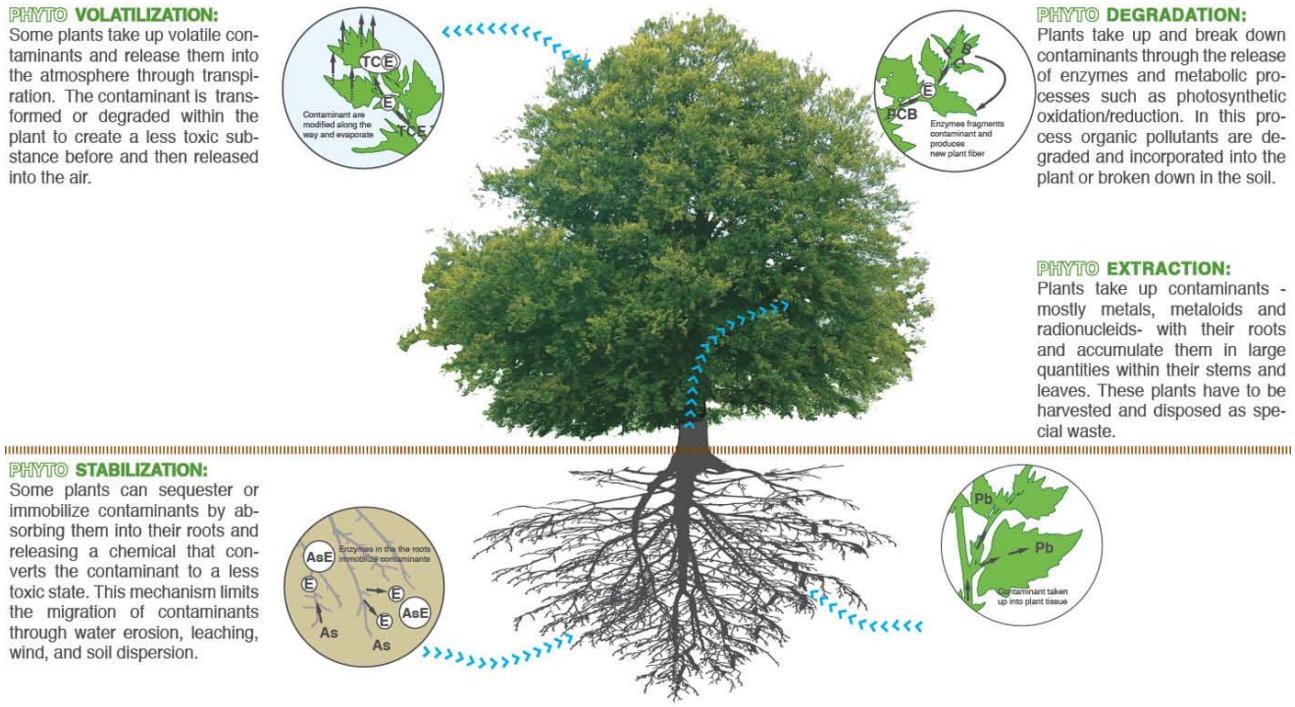

Figure 1.5: The overall name of "phytoremediation" gathers different physiological processes.
URL: http://urbanomnibus.net/2010/11/from-brownfields-to-greenfields-a-field-guide-to-phytoremediation/

This relatively new emerging technique is seen with interest by the world of research because is a promising cleanup solution for a wide variety of pollutants related to different anthropogenic activities (i.e. agriculture, chemical industry, mining extraction, landfills, sewage treatment and urbanization). Currently, phytoremediation is used for treating many classes of contaminants including petroleum hydrocarbons, chlorinated solvents, pesticides, explosives, heavy metals and radionuclides, and landfill leachates. Plants used for phytoremediation should be fast growing, deep-rooted, easily propagated and accumulate the target pollutant (Robinson et al., 2000), and black poplar exhibit these properties making it good candidate for phytoremediation. It is clear how the success of remediation is strictly linked to biomass productivity of the plantation, so over the years a great number of studies focused attention on hybrid poplars, such as *Populus* × *canadensis* Moech., natural cross between *P. nigra* and *P. deltoids*. Bioaccumulation ability of *P.* × *canadensis and P. nigra* was tested for a lot of different pollutants; the suspected carcinogen 1,4-dioxane (Aitchison et al., 2000), for cadmium used in fertilizers (Gaudet et al., 2011; Zacchini et al., 2011, Robinson et al., 2000), for the high explosive 2,4,6-trinitrotoluene (TNT) (Thompson et al., 1998), for atrazine used in pest control (Chang et al., 2005) and many volatile compound (Burken and Schnoor, 1999).

Not less important of the aforementioned uses, poplar utilization contributes also to carbon sequestration. The need for substantive CO_2 emission reductions could be satisfied more cheaply through available sequestration technologies than by an immediate transition to nuclear, wind or solar energy (Lackner, 2003). An effective role may be envisioned for black poplar and its hybrids in carbon dioxide (CO_2) sequestration schemes, thanks to the main characteristics of these species (i.e. fast growth, adaptability to different environmental conditions and suitability for diverse silvicultural systems). Plantations and natural stands of poplar, are efficient sinks of CO_2, allocating captured carbon between belowground and aboveground biomass (Isebrands and Karnosky, 2001). Ultimately, poplar plantations provide a wide mitigation strategy in response to global warming, so for this reason some studies evaluated how management practices and plant materials affect the CO_2 sequestration. For example, was observed that rural windbreak of poplar, with 2.5 m spacing between trees, would have an average above ground biomass of 174.8 Mg per km and would contain 84.2 Mg of carbon (Kort and Turnock, 1996). Fang et al. (2007) for a 10 years-old stand with 1111 trees per hectare, observed a total biomass production of 146 Mg per hectare that are equivalent to 72 Mg per hectare of stored carbon.

Despite the environmental advantages generated by natural populations of *Populus nigra*, the decrease of natural range of black poplar and the decline of its genetic variability are present problems of riparian forests management. The habitat integrity of this species is threatened by urbanization of riparian ecosystems, with subsequent reduction of its natural range. Furthermore, introgression of exotic poplar genes into natural populations, due to intensive breeding activity with alien poplar species, genetic integrity of black poplar is seriously threatened (Vanden Broeck, 2003). Many forest biologists regard *P. nigra* to be one of the most threatened tree species in Europe (Dickmann and Kuzovkina, 2008), in fact the riparian forests of *Populus* and *Salix* are designated priority habitat in Europe's Nature 2000 conservation strategy (Directive 92/43/EEC). In order to protect poplar geographic distribution and its genetic pool, inventory and protection of extant natural *P. nigra* populations are being employed. Since 2008, the European Forest Genetic Resources Programme (EUFORGEN) coordinates these efforts in the European Union.

1.1.4. Economic interest of Populus nigra L.

Populus nigra is a tree of particular economic interest. It is a crossing parent in the breeding of healthy and fast-growing *Populus* × *canadensis* Moench (*P. deltoides* × *P. nigra*), hybrids which are grown widely in Europe and in other parts of the world (Vanden Broeck, 2003). Presently, the worldwide *Populus* estate encompasses over 5255000 hectares of plantations and 3867000 hectares

of agro-forestry and environmental plantings (FAO, 2008). A surface of 940200 hectares addressed to poplar's plantations is estimated, equal to 4% of the total plantations present in Europe. Wood production remains the main purpose for poplar growing in European Union, accounting for 855000 hectares (90%). Some 80000 hectares (8%) of poplars are reported as being used for various protective systems. The poplar roundwood available annually amounts to an average of 8045000 cubic meters, destined to the production of plywood and veneers (40%), sawn-wood (31%), pulp paper (15%), reconstituted wood (9%), bioenergy (4%) and other uses (1%) (Coaloa and Nervo, 2011). In Italy 118500 hectares are filled by poplar plantations, that is the 12% of the total European distribution. In the same country 20% of poplar plantations (23700 hectares) have a protective purpose, while other 80% (94800 hectares) have a productive purpose (Coaloa and Nervo, 2011). "L'Inventario Nazionale delle Foreste e dei Serbatoi Forestali di Carbonio" (INFC, 2007) provides lower values, with only 66.269 hectares used in poplar arboriculture. In Italy, the poplar roundwood available annually amounts to closed values of 868000 cubic meters (Coaloa and Nervo, 2011) and 994168 cubic meters (INFC, 2007), destined to the production of sawn-wood (65%), pulp paper and plywood (33%), and other uses (2%) (INFC, 2007).

Figure 1.6: Traditional poplar plantation (on the left) and harvest of a poplar's short-rotation coppice culture (SRC) (on the right).

Nowadays, poplar have became of interest as a potential source of sustainable and renewable biomass for the bioenergy, biofuel and bioproduct industries. According to the purposes of "The 2020 climate and energy package" (Directive 2009/29/EC), short-rotation coppice cultures (SRCs)

(Fig. 1.6) can be expected to play a major role as a renewable energy source, in order to substitute fossil fuels and to reduce greenhouse gas emissions . The main goals of this directive to achieve until 2020 are: (i) the reduction of total amount of greenhouse gases emission produced by 20% respect to baseline of 1990, (ii) the rising at 20% of total energy requirements the amount of energy derived from renewable sources, (iii) reduction of utilization of primary (fossil fuels) energy sources by 20%. Thanks to the rapid juvenile growth and response to cultural treatments, the easy production of hybrids and the easy vegetative propagation, poplar's hybrids are ideal for short-rotation intensive culture (Wyckoff and Zasada, 2007). Since wood products are a renewable and relatively energy efficient source of material, can be reduced by using timber in place of more energy-intensive resources. Moreover, the new interest in poplar as an industrial crop encouraged the initiation of new breeding programmes. As with all crops, emphasis was placed on achieving yield gains through both agronomic and genetic improvements.

1.1.5. Scientific interest of Populus genus.

The biology of woody plants provides an opportunity to study processes for which herbaceous models are less well suited. Tree-specific traits such as wood formation, long-term perennial growth, and seasonality are obvious areas of research, but research in other areas such as control of flowering, biotic interactions, and evolution of adaptive traits, is enriched by adding a tree to the suite of model systems (Jansson and Douglas, 2007). Generally, relatively high levels of genetic and phenotypic diversity (Dickmann, 2001) make *Populus* genus an excellent model system for studying evolutionary processes such as adaptation. The great diversity of species distributed on a worldwide scale at different elevation and latitude ranges (Brunner et al., 2004), extensive species variations in natural populations and the plasticity observed in different environments (Jansson and Douglas, 2007), the wide degree of inter-specific hybridization that occurs within and between sections together with capacity of asexual propagation (Stettler and Bradshaw, 1996),all these attributes were the groundwork that led to consider *Populus* a representative model of all vascular land plants. Moreover, rapid growth rate and ease vegetative propagation allow researchers to quickly set trials and to collect data, in order to measure short-term responses to abiotic and biotic factors (Bradshaw et al., 2000 ; Dickmann, 2001). A crucial trait that made *Populus* a species of primary interest for the scientific comunity, is the small genome size with a haploid chromosome number of 19 and about 500 million base pairs (Bradshaw et al., 2000). In fact, *Populus* genome is 40 times smaller than size of *Pinus* genus (about 22 billion base pairs) and is clear like genomic and molecular analyses (i.e. genome sequencing) require lower time and economic efforts for the former

genus. In the last decennium, rapid development of genomic, molecular biology and bioinformatics resources occurred. Publicly accessible DNA sequences, DNA microarrays, expressed sequence tags (ESTs) collections, bacterial artificial chromosome (BAC) library, high-throughput plant transformation and regeneration capabilities and gene ontology (GO), have been developed for *Populus*. Since September 2004, the entire genome sequence of *Populus trichocarpa* Torr. & Gray (Tuskan et al., 2006) is available on the website of Joint Genome Institute (http://jgi.doe.gov/). The *P. trichocarpa* genome sequence provides a powerful tool for exploring genome structure and function in poplar and in analogous genus using comparative mapping.

1.2. Growth related traits

1.2.1. A general overview

Tree growth is determined by cell division and expansion in the apical and cambial meristems, developmental and seasonal growth transitions, efficiency of photosynthesis, nutrient and water uptake and transport, and the ability to respond to biotic and abiotic stresses (Grattapaglia et al., 2009). Hence to consider the growth of a plant as a simple increase of size is to oversimplify an articulate process. Growth related traits in trees are fundamental components of survival and productivity in natural and planted forests. The overall category of "growth related traits" refers to many different attributes: morphologic traits, such as overall size, crown architecture, biomass allocation (among roots, stem, branches and leaves) and growth rate, and physiological activities, as well meristems development (buds, roots and fruits), nutrients uptake from soil, water use efficiency and secondary metabolites production and use (i.e. alkaloids, terpenoids and flavonoids). Moreover, the tree's adaptation capacity to changeable abiotic conditions (i.e. drought, hypoxia, high salinity and coldness) and to synergistic or antagonistic biotic interactions (i.e. pests, fungus, and bacteria), is a critical characteristic for survival and competitive success of woody plants. As reaction to changes of "standard" background, organisms respond by tracking the environment and/or showing particular performances (i.e. plasticity) to new environmental combinations. It is possible, such as extreme form of adaptation, that new combinations of standing genetic variation or new genetic mutations generate novel adaptive genotypes (Olson et al., 2013).

All these functional traits are objectives of study in a large number of scientific works. As example of hundreds of work concerning growth traits are mentioned: Deinlein and coworkers (2014) highlighted the HKT genes as a major player in plant salt tolerance and root-shoot Na^+ partitioning, Nunes-Nesi and coworkers (2014) focused the attention on the complex role of mitochondrial

metabolism in plant aluminum resistance and Sozzani and coworkers (2013) emphasize how advanced imaging techniques procure multi-level data for investigation of plant growth and development. It is apparent that understanding tree growth, in all its ”meaning”, will have a significant impact on the management of adaptive genetic variation needed for forest survival in changing environments. Furthermore, from a utilitarian perspective, the efficiency of selective breeding for genetic gain in wood productivity is another main target (Grattapaglia et al., 2009).

In particular, aboveground biomass allocation and bud flush phenology were objectives of great interest by plant ecologists and breeders. Through the establishment of scientific trials and focused breeding programs, the study of natural different populations or specific families could lead the screening of most promising genotypes. Understanding the genetic basis and environmental influence for these traits is a key point concerning a global strategy of natural ecosystem conservation and carbon-neutral renewable energy production.

1.2.2. Aboveground biomass production and allocation

Worldwide, forests represent half of the biospheric carbon sink. It is estimated that forest biomass is gaining more than 2 Pg C (2×10^{15} grams of carbon) each year (Pan et al., 2011) due to increased number and growth of individual trees. However, a correct management of this huge resource is necessary, in light of impending water and arable land shortages and climate change, to sustainably meet future demands for wood, biomass, paper, fuel and biomaterials. Hardwood trees provide a sustainable source of carbon-neutral bioenergy able to combat the effect of rising atmospheric CO_2 and other greenhouse gases (Tuskan 1998, Sims et al., 2006). The increasing importance of bioenergy in the present geopolitical and environmental context has aroused renewed interest in tree cultivation under short-rotation regimes in Europe. *Populus* hybrids are superior hardwood trees for biomass production (Rae et al. 2004), producing yields of up to 35 oven-dried tonnes per hectare per year (t ha^{-1} $year^{-1}$) (Scarascia Mugnozza et al. 1997). Within the *Populus* genus, a large phenotypic as well as genetic variation in growth performance, canopy architecture and physiology is available (Ceulemans et al. 1990, Cervera et al. 2005), with remarkable plasticity for plant productivity estimated in different environments (Marron et al., 2010a). Moreover, very high plasticity was found in the allocation of dry mass among leaves, stems and roots (Poorter and Nagel, 2000; Marron et al., 2010b).

Tree growth can be defined as the increase in dimensions of an individual tree through time. The most commonly measured dimensions are height and circumference, because these are convenient

measures that are strongly correlated with wood volume and weight. These direct indicators of growth have demonstrated their efficiency as selection criteria (Marron et al., 2006) and in allometric equations used to indirectly estimate tree biomass or volume (Dillen et al., 2007). Moreover these more easily measured traits, shoot branching plays a pivotal role in the development of the aboveground structure and in determination of growth performance of tree species used in dense commercial plantations. Sylleptic branches, that are branches developed from a bud without undergoing a dormant season (Remphrey and Powell 1985), are important as they are intimately associated with tree architecture and growth, and because they translocate a larger proportion of carbon to the stem than proleptic branches during the early growing years of poplar trees (Ceulemans et al. 1990; Scarascia-Mugnozza, 1991). Shoot branching is highly plastic, because of sensitivity to environmental factors (i.e. light signalling and intensity), and it is actively manipulated through pruning and harvesting: removal of main branches induces outgrowth of dormant axillary buds, creating new branches (Evers et al., 2011). To the other side, hormone signalling network (auxin, strigolactones and cytokinin) exercises an extended internal control on branches development (Leyser, 2009).

The prediction of aboveground biomass production is indispensable for the market development of any agricultural or forestry crop. Biomass estimation procedures in short-rotation forestry differ in their methodology, complexity and time demand depending on the specific goal of the estimation strategy (Verwijst and Telenius, 1999). Methods of indirect estimation have been developed, because are preferred to direct measurements of biomass, which are destructive and time consuming activities (Ketterings et al., 2001). The main objective of indirect methods is to estimate biomass of many trees through sampling a low number of individuals, in order to catch the genetic variation available within genotypes, families or whole populations. Among the different methods for the indirect prediction of aboveground biomass production, the regression method is the most frequently used. A multiple regression model establishes an equation relating aboveground tree biomass to the dimensions (stem circumference, diameter or height) and architecture (ramification) of the tree. However, the equations resulting from the regression analysis may vary in terms of parameters of the model and/or model form (linear versus nonlinear) as the equations reflect the population and its characteristics (i.e. genotype, age, environment, plant density and plantation management) on which they were developed (Verwijst and Telenius, 1999; Wang and Kimmins, 2002; Zabek and Prescott, 2006).

In summary, understanding the environmental and genetic basis of traits linked to biomass production and improving the commercial biomass yield of hybrid poplar are essential requirements

for short-rotation forestry to became an economically viable alternative to other land uses (Updegraff et al., 2004).

1.2.3. Plant phenology and global warming

Phenology is the study of recurring events that annually occur during the life cycle of plants and animals (Kramer, 1996), as a result of natural selection, and how they respond to seasonal change in their environment. Phenology is the macroscopic manifestation of fundamental endogenous processes of an organism, which is influenced by environmental factors, such as climate, soil and silvicultural practices. The study of plant phenology is supported by other sciences, such as ecology, climatology, physiology, genetics, and molecular biology in order to explain the phenomenon. In the century agricultural sciences showed deep interest for phenology, to determine the period of major vulnerability to drought, coldness and pests, and the effects on the growth during the season, with the general aim to maximize the crop yield. The longest and best known phenological records come from the Far East and Europe, including a 5000-year record of phenological events, weather and farming activities in China (Chen, 2003) and the 670 years of grape harvest dates in Central Europe (Chuine et al., 2004). Presently in a context of ecosystem and biodiversity preservation, changes in plants phenology are observed in function of climate alteration and global warming.

In the context of a sessile existence, plants must adapt themselves to seasonal changes of conditions, synchronizing the annual growth cycle with this variability (Ruttink et al., 2007). In equatorial areas, the lack of seasonal variability of temperatures allows plants to grow indefinitely throughout the year, conversely growth is restricted to a specific period (i.e. growing season) in the boreal zones. For this reason, trees such as poplars have evolved a mechanism whereby they can switch from active growth during the favorable season to dormancy in rest season, in response to the environmental signals. At the same times in response to other or the same inputs, perennial plants can release the dormancy status and to start a new growing season with the next spring (Fig. 1.7). Buds' phenology is the results of this adaptation.

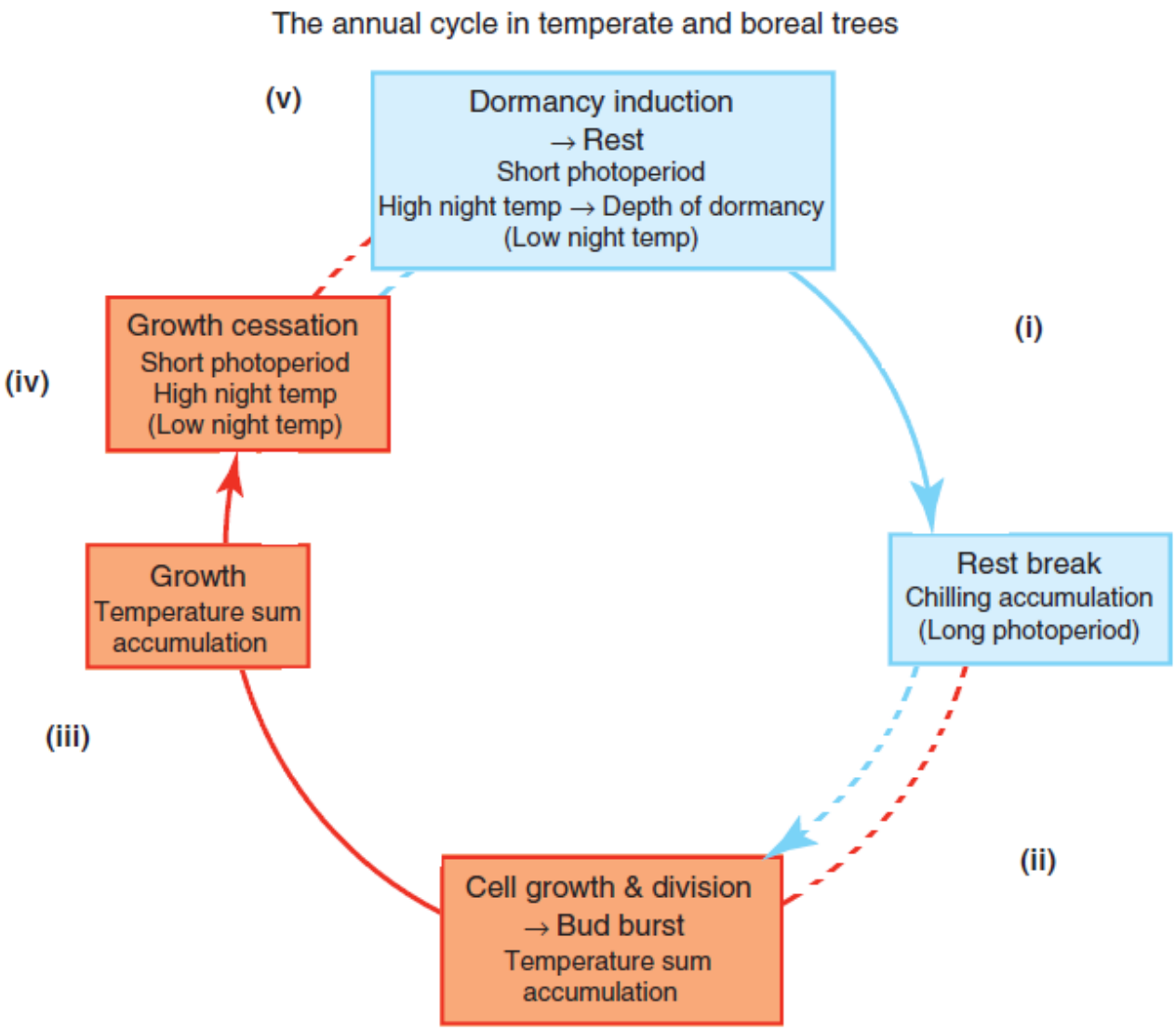

Figure 1.7: Annual cycle of growth and dormancy in boreal and temperate trees, identifying the ecophysiological phenomena that determine the timing of growth onset and cessation. Periods of growth (red arrows) and dormancy (blue arrows) are shown. The alternation between growth and dormancy occurs gradually (dashed arrows). (i) Long-term accumulation of chilling during late autumn and early winter causes rest break. (ii) After rest completion, growth onset (e.g. bud burst) is brought on by the cumulative effects of high temperatures. Temperature sum accumulation regulates height growth and, in tree species with a fixed growth habit, also its cessation. (iv) In most species with a free growth habit, a short photoperiod is an essential environmental cue inducing growth cessation (e.g. bud set). (v) Dormancy is induced by short photoperiods. (Hänninen and Tanino, 2011. Trends in Plant Science, Volume 16, No. 8, pp. 412-416).

Phenological changes have now been widely linked to recent global warming in a range of plants (Menzel et al. 2006), range limits that are repeatedly reshaped (Umina et al., 2005; Saccheri et al., 2008). Altered phenological patterns generated by climatic changes can have great influence on carbon cycle (Leinonen and Kramer, 2002), plants ecology (Morin et al., 2008) and their herbivores (Visser and Holleman, 2001). Key phenological shifts detected in plants include earlier budding, flowering time and seed maturation under warmer and drier conditions (Root et al. 2003; Menzel et al. 2006). These shifts in phenology can influence the fitness of plants directly or indirectly through interactions with other organisms. Furthermore, in trees the activity of the bud determines not only the extent of seasonal growth, which largely determines tree productivity and wood quality in temperate and boreal zones, but also growth habit and tree form (Rhode et al., 2000). Following shoot design of apical meristem implanted by vascular plants are shown (Fig. 1.8) (van der Schoot and Rinne, 1999).

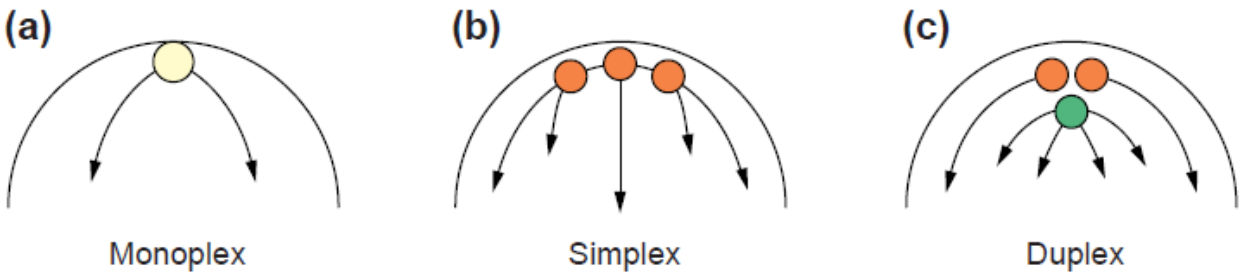

Figure 1.8: Types of shoot apical meristem (AM): (a) the monoplex; (b) the simplex; (c) the duplex which are characteristic of the majority of ferns and lower vascular plants, gymnosperms and angiosperms, respectively. (a) The monoplex AM has a single top cell, which is often tetrahedral and produces daughter cells by lateral division. (b) The simplex AM has a zone of initial cells in an unstable superficial layer, where cells can divide in both directions. (c) The duplex AM has two distinct zones of initial cells which give rise to two lineage compartments, tunica and corpus. (van der Schoot and Rinne, 1999. Trends in Plant Science, Volume 4, No. 1, pp. 31-37)

The timing of bud flush during the spring and bud set in the fall represents a critical ecological and evolutionary trade-off between plant growth and survival (Horvath et al., 2003; Howe et al., 2003). A perfect synchronization between development of trees and annual temperature cycle is essential for biological success of plants. In the recent decades, the main research focus has been on the acceleration of springtime phenological events of plants (e.g. bud burst in boreal and temperate trees, such as poplars), which can be caused by climate warming (Kramer et al., 2000; Beaubien and Freeland 2000; Menzel et al., 2006; Linkosalo et al., 2009). Early onset in springs and late cessation of growth in fall, resulting in exposure of vulnerable plant tissues and organs, give rise to frequent

frost damage. On the other hand, late onset and early cessation result in loss of growth resources (Partanen et al., 1998). Hence, to find the right compromise solution between the necessity to avoid the risk of frost damage and assure the maximization of the growth season, is crucial for plants such as Populus which show a continuous development during the growing season (indeterminate growth) (Luquez et al., 2008). However, recent studies showed that interactions between plants' genotype with chilling temperatures and photoperiod work as natural mitigation response to phenological shifting, and also, whether bud flush and growth onset are accelerated, the acceleration response can be non-linear to increased levels of warming (Körner and Basier, 2010; Morin et al., 2010). Moreover, Tanino and coworkers (2010) found that rising temperatures move up, rather than delay, the growth cessation. A physiological basis links rising air temperatures with cell division and growth in the buds, but this simple relationship might be complicated in several ways by the release of dormancy. A time of exposure to chilling temperatures (i.e. chilling requirement) is needed to remove deep dormancy and break the rest status. By the 1989, Murray and coworkers hypothesized a delay of bud flush date for some temperate species such as *P. trichocarpa*, due to delayed chilling requirement caused by milder winters. In trees with a low chilling requirement, warming accelerates bud burst more than in trees with a higher requirement (Morin et al., 2010). Moreover, there is increasing experimental evidence that elevated air temperatures during dormancy induction in late summer and early autumn increase the depth of dormancy, so that more chilling is required for rest break and/or more accumulation of temperature sum for bud burst (Tanino et al., 2010; Heide, 2003; Granhus et al., 2009). Thus, besides the impact of reduced chilling being carried over from the previous autumn and winter, the impacts of elevated air temperatures, with potentially delayed bud burst in spring, could be carried over from an even earlier time (i.e. from the previous summer) (Hänninen and Tanino, 2011).

It is generally accepted that rest break occurs when buds are exposed to low temperature (below 0°C) for several weeks (Fuchigami et al., 1982, Cannel and Smith, 1983), and the same idea is accepted for bud flush, that occurs when buds are exposed for a prolonged period to temperature above a certain threshold (Fuchigami et al., 1982, Cannel 1990). Hence, over than simple chronological records of the bud flush (i.e. the day of the year of the onset of the process), thermal parameters are used to study the phenomenon. A large use of daily average temperatures was made in bibliography to describe influence of temperatures on bud flush beginning and dynamics (Partanen et al., 1998; Pellis et al., 2004; Olson et al., 2013, Ghelardini et al., 2014). The growing degree-day (GDD) concept is based on a linear relationship between temperature and developmental rate of the plant (Baker et al., 1984. According to this concept, every stage of the life

cycle of a plant can be presented by a temperature sum (degree-day sum), calculated according to this general formula (Pellis et al., 2004):

$$\mathrm{GDD} = \sum (\mathrm{T_m} - \mathrm{T_b})$$

where,

T_m: mean daily temperature,

T_b: base or threshold temperature.

It is evident that a start day is required to accumulate temperature and estimate GDD. As regards to studies performed on bud flush, the starting date of the degree-day summation and of the base temperature were chosen arbitrarily, often namely 1 January and 0°C, respectively. However beyond these general statements, a large number of studies performed on more species, in different conditions, with different methods, suggest that threshold temperature and timing necessary are species and environment specific.

1.3. Quantitative genetics

The study of polygenic traits, called quantitative genetics, involves partitioning (i.e. subdividing) the measured phenotypic variation in natural or breeding populations of forest trees into the portions caused by genetic and environmental influences. Characteristics such as bud development and tree dimension are known to be typical quantitative traits, and their study is very important because they are adaptive and economic traits. Main characteristics of polygenetic traits are listed (White et al., 2007):

- Each single gene locus has a small, unobservable effect on phenotypic expression of the trait,
- At a given locus, each alternative allele has a small effect on phenotypic expression,
- The environment in which a tree grows also affects the phenotypic expression, confounding even more the ability to observe genetic differences directly,
- Phenotypic differences usually occur on a continuous gradient,

- Individuals with the same phenotype almost always have different genotypes, differing perhaps in specific alleles at many loci,
- The number of loci controlling the trait and the allele frequencies at those loci are almost never known.

The combination between genetic potential of a generic individual (i.e. genotype) and the effects exercised by the environment in which the tree is growing is the phenotype (Zobel and Talbert, 1984). Hence, the phenotype is the real manifestation of the phenomenon, and is the thing directly observed in field, that is the data collected and later analyzed.

Because it is not possible identify specific genotypes nor allocate them into specific genotypic classes for polygenic traits, statistical methods are used to quantify the amount of phenotypic variation for each measured trait, and to estimate the relative contributions of different types of genetic and environmental influences to this observed phenotypic variation. Descriptive statistic parameters like population mean (μ), standard deviation (σ), variance (σ^2) and coefficient of variation (CV) are basic instruments to describe quantitative traits. However, these instruments are not sufficient to point out the relative importance of different sources of variation (i.e. genotype, sites, years, etc.) when operate, at the same time, to define the phenotypic variance. Analyses of variance (ANOVA) is powerful tools provided by inferential statistics, that can achieve objectives can not be reached by simple descriptive parameters. It is used to analyze the differences between group means ("variation" among or between groups). In the ANOVA setting, the observed variance in a particular variable is partitioned into components attributable to different sources of variation, based on a linear regression model that links the phenotypic values with source effect:

$$P_i = \mu + G_i + E_i$$

where,

P_i: is the phenotypic value of i^{th} tree,

μ: is the population mean,

G_i: is the underlying genotypic value of i^{th} tree,

E_i: is the cumulative effect of all environmental influence of i^{th} tree.

Since the mean, μ, is a constant, the equation specifies that a tree's phenotypic measurement is controlled by two underlying variables (G_i and E_i), and these two underlying variables are confounded. That is, for any number of phenotypes measured, there is twice the number of unknowns as measurements; therefore, G_i and E_i cannot be separated. For this reason, geneticists must plant progeny (either clonal or sexual offspring) in randomized, replicated trials to separately measure genetic and environmental effects on phenotypes (White et al., 2007).In quantitative genetics, the relative importance of genetic and environmental influences is quantified in terms of their contribution to the observed phenotypic variance. With fixed values μ, its variance is null, hence:

$$\sigma_P^2 = \sigma_G^2 + \sigma_E^2$$

where,

σ_P^2: total phenotypic variance,

σ_G^2: variance due to genotypes,

σ_E^2: variance due to environment effects.

For example, if all observations are from the same clone (i.e. have the same genotype), then does not exist genetic variability ($\sigma_G^2 = 0$), and the phenotypic variance is totally due to environment effects ($\sigma_P^2 = \sigma_E^2$). Conversely, if the environment is completely uniform, such that all trees experience the same microenvironment ($\sigma_E^2 = 0$), only the individual genetic variation contributes to phenotypic values ($\sigma_P^2 = \sigma_G^2$).

These extreme examples are meant to illustrate that it is possible to quantify the relative importance of genetic and environmental variability in terms of their respective contributions to the phenotypic variance. The heritability is the parameter that summarizes the relationship between sources of variance. Broad-sense heritability (H^2) is defined as the ratio of total genetic variation in a population to the phenotypic variation and it can be written as:

$$H^2 = \frac{\sigma_G^2}{\sigma_P^2}$$

The broad-sense heritability limits are 0, when none of variation in a population is due to genetics, and 1, when we have not environmental effects on the variation. An important but often underestimate aspect of heritability is that they applies only to a particular population growing in a particular site at a particular moment because a generic quantitative trait may not be influenced by exactly the same genes when grow in different environmental or experimental conditions. The heritability value of a given trait also change with age when the environment change and when the genetic control of the character change as the tree mature (Zobel and Talbert, 1984).

However, the described model can be considered a simplification of the more articulate situation that occurs in nature. In fact in this simplify model is not considered an important source of variation, the genotype by environment interaction (G×E). The essence of G×E interaction is a lack of consistency in the relative performance of genotypes when they are grown in different environments (Barcaccia and Falcinelli 2005). This may mean that the relative rankings of genotypes change in the different environments (called rank change interaction) or that, even in the absence of rank changes, differences in performance are not constant in all environments (called scale effect interaction). The phenotypic response to the environment can be expressed in so-called "reaction norms" (Fig. 1.9) and G×E occurs when the slope of the reaction norms differs for different genotypes (El-Soda et al., 2014).

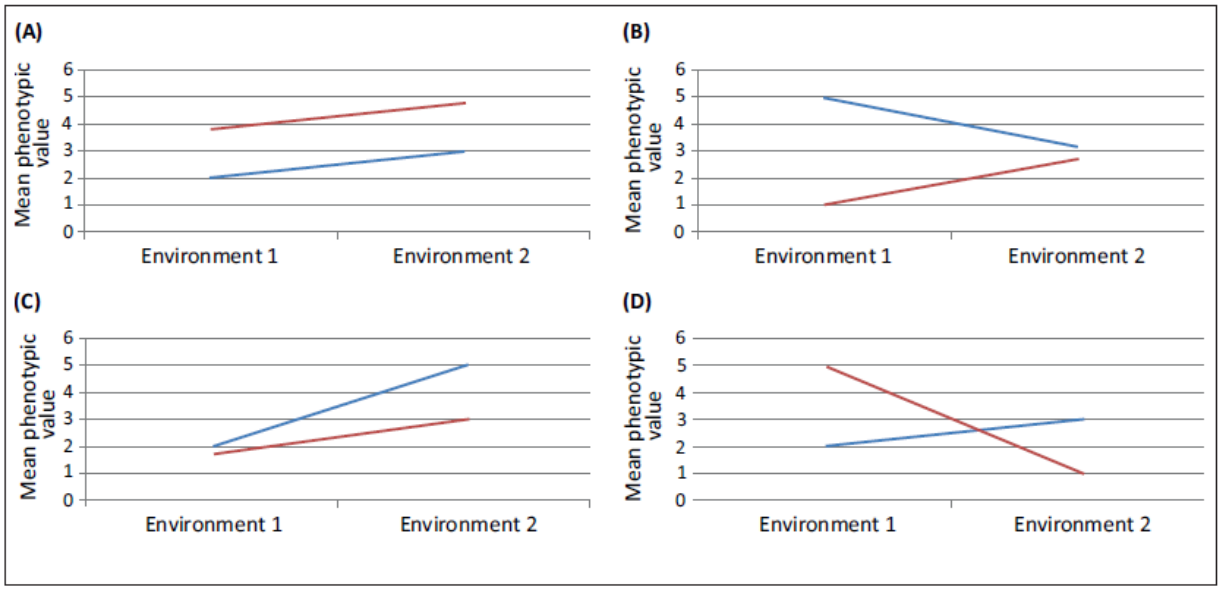

Figure 1.9: Genotype by environment interaction and reaction norms. (A) The trait values change across environments but the reaction norms run parallel because the response to the environmental conditions is similar for both genotypes (red and blue lines). There is a scale-effect interaction indicating G×E, because each genotype has a different response to each of the environments, but without the reaction norms to cross (B and C). Whereas, there is a stronger genetic effect on the phenotype in response to the different environments, also indicating G×E, causing reaction norms to cross (D). (El-Soda et al., 2014. Trends in Plant Science, paper in printing).

Basing on abundant bibliography about G×E, the mechanisms explaining genetic basis of this effects are following summarized (El-Soda et al., 2014):

- Overdominance: plasticity is a function of homozygosity, with phenotypic plasticity being negatively correlated with the number of heterozygous loci,
- Pleiotropy or allelic sensitivity: a pleiotropic gene affects two plastic traits and may have different effects on both traits in different environments,
- Epistasis: loci producing plasticity may modify the expression of other genes to be turned on or off in an environment-specific fashion,
- Genetic linkage: alleles promoting plasticity may be linked with alleles conferring either low or high fitness,
- Epigenesis: the impact of chromatin modification and DNA methylation may depend on the environment and can lead to epigenetic changes in gene function and, consequently, changes in the phenotypes, without changing the DNA sequence.

Statistically, G×E interaction is a two-way interaction in an analysis of variance that occurs when true differences between genotypes for any trait are not constant in all environments in which they are planted. The linear model aforementioned defined is no longer adequate to explain the observed variation, so a new term must be added to equation ($\sigma^2_{G\times E}$):

$$\sigma^2_P = \sigma^2_G + \sigma^2_E + \sigma^2_{G\times E}$$

where,

$\sigma^2_{G\times E}$: variance due to interaction term.

It can be of great benefit when maximum gains are desired in specific environment, but the G×E interaction can became a formidable barrier when an environmental stable genotype for a given trait is sought (Zobel and Talbert, 1984). Evaluating genotypes in multiple environments is essential to gain insight into the extent of G×E and this is of great interest for crop breeders to assess how much of the selection progress achieved in one environment can be carried over to other environments. A

good method to reduce the G×E interaction in breeding program is to select for genotypes that perform well and that show little interaction over different environmental condition; for this aim several statistics estimating stability (Wricke, 1962; Gauch, 1992, Brancourt-Hulmel et al., 1997,) have been developed to understand which genotypes mainly contribute to the total G×E interaction in a given population studied. The G×E source of phenotypic variability is considered very important and an understanding of the genetic control of this interaction is of great importance for quantitative genetics and its applications in breeding, conservation and theory of evolution (Rae et al., 2008).

1.4. Quantitative traits loci (QTLs)

Since the proposal of the multiple-factor hypothesis by Nillson-Ehle in 1909, the genetic variation of a quantitative trait is assumed to be controlled by the collective effects of numerous genes, known as quantitative trait loci (QTLs). The QTL mapping techniques were developed in the late 1980s with the advent of molecular markers and it allows us to dissect quantitative variation and understand the genetic control of such complex traits. The two general goals of QTL mapping in plants are to increase our biological knowledge of the inheritance and genetic architecture of quantitative traits, both within a species and across related species, and identify markers that can be used as indirect selection tools in breeding (Bernardo, 2008). Ultimately, mapping QTLs makes possible the manipulation of genes in breeding programs.

Quantitative trait locus analysis is a statistical method that links two types of information: phenotypic data (trait measurements) and genotypic data (usually molecular markers), in an attempt to explain the genetic basis of variation in complex traits (Falconer & Mackay, 1996; Lynch & Walsh, 1998). Markers that are genetically linked to a QTL influencing the trait of interest will segregate more frequently with trait values, whereas unlinked markers will not show significant association with phenotype (Miles and Wayne, 2008). The number of QTLs detected in a given study depends on different factors, including type and size of mapping population used, trait investigated, the number of environments used for phenotyping, and genome coverage.

In 1989, Lander and Botstein developed the method which has become the standard technique used by many geneticists for mapping QTL, the simple interval mapping (SIM). Once a linkage map and phenotypic data are available for a population, SIM uses one marker-interval at a time to search for a hypothetical QTL (the target QTL) by performing a likelihood ratio test at every position within the interval. In this approach, the QTL is located within a chromosomal interval, defined by the flanking markers. Lander and Botstein (1989) proposed a simple rule for constructing confidence

intervals for QTL position, which uses the likelihoods of odds (LOD score). LOD score is the base-10 logarithm of the ratio of two likelihoods (probabilities): the likelihood of the observed data assuming a QTL at the position in question and the likelihood assuming no QTL. The results of the analysis are plotted as a LOD score against the chromosomal map position in cM. The chromosomal location of the maximum LOD score is taken as the position of the QTL (Fig. 1.10).

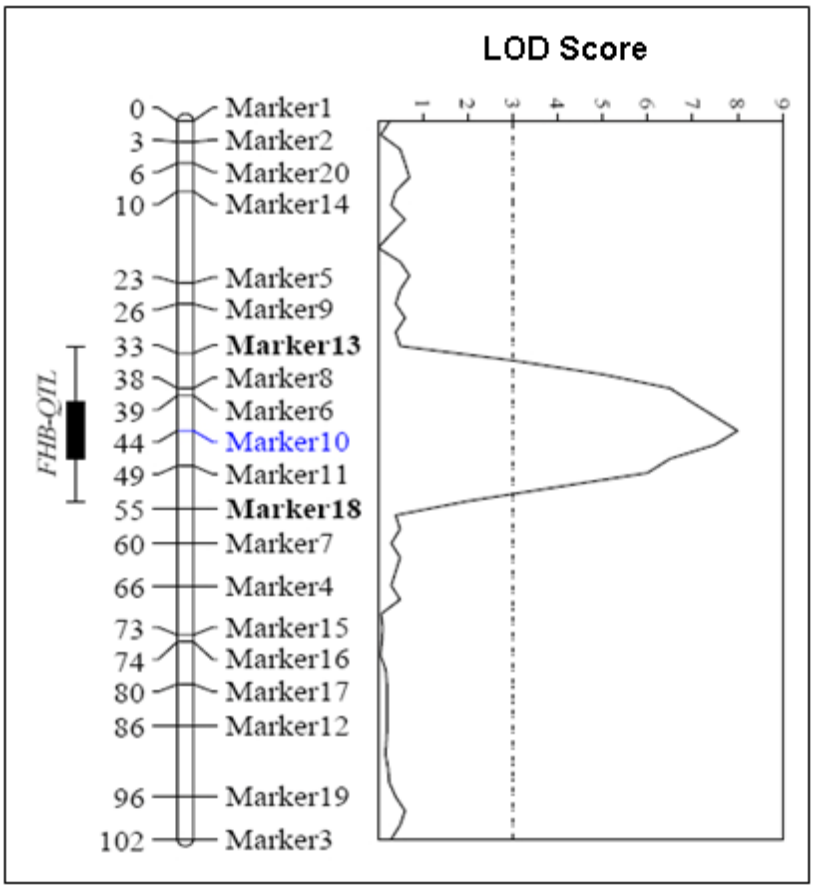

Figure 1.10: The interval mapping approach for QTL mapping. The results of QTL mapping are plotted as a likelihood-ratio test statistic (LOD score) against the chromosomal map distance, measured in recombination units (centiMorgans). The vertical dotted line represents a threshold value above which a likelihood-ratio test provides a statistically significant fit to a model of the data. The best estimate of the location of the QTL is given by the chromosomal location that corresponds to the highest LOD score. On the left of linkage group, the middle segment (black bar) on the line indicates the peak of the QTL, and the total length of the line represents the 95% confidence interval. In this hypothetical example, maximum LOD score is at 44 cM and the confidence interval is between 36 and 54 cM. Marker10 is the closest marker to the QTL while Marker13 and Marker18 are the two flanking markers. (Semagn et al., 2010. Electronic Journal of Biotechnology, Volume 13, No. 5, pp. 1-45).

However, the SIM model shown three principal limitation (Semagn et al., 2010):

- Simple interval mapping considers one QTL at a time in the model (single-QTL model), ignoring the effects of other QTLs,
- QTLs outside the interval under consideration can affect the ability to find a QTL within it,
- False identification of a QTL can arise if other QTLs are linked to the interval of interest.

Multiple-QTL models are an improvement over single-QTL models because of their ability to separate linked QTLs on the same chromosome and to detect interacting QTLs that may otherwise be undetected (Schork, 1993). To address the limitation of SIM, Kao and coworkers (1999) proposed and implemented multiple interval mapping (MIM) for mapping multiple QTLs simultaneously. The idea of MIM is to fit multiple putative QTL effects and associated epistatic effects directly in a model to facilitate the search, test and estimation of positions, effects and interactions of multiple QTLs.

Summarizing, the MIM QTLs analyses provides information on (Semagn et al., 2010):

- the number and chromosomal location of QTLs affecting a trait,
- the magnitude and direction of the effect of each QTL (i.e., whether a phenotypic trait is controlled by many genes or many independent loci of small effect or by a few genes of large effect),
- the mode of gene action at each QTL (dominant or additive),
- the parental sources of beneficial QTL alleles,
- whether there is interaction between different QTLs (i.e. Epistasis) or between genotypes and environment.

When a QTL is found, it is often not the actual gene underlying the phenotypic trait, but rather a region of DNA that is closely linked with the gene. With the availability of the entire genome sequence of *Populus trichocarpa*, it is possible to link the QTLs found on a genetic map (density of recombination) with the genome physical map (real position on a chromosome), hence to check possible expression by “candidate genes” for the traits linked to these QTLs. Moreover, QTLs detected on more genetic maps of related species (i.e. species of the same genus) can be analyzed through technique of comparative mapping, in order to identify inversions, translocation and duplications that have occurred in the DNA sequences. Genetic factors can also be assessed by comparing map distances of genes with conserved gene order in the two species.

2. OBJECTIVES

Climate change impacts on plant phenology have attracted much attention (Olson et al., 2013; Walther et al., 2002), partly because of the consequent effect on population and species distributions (Umina et al., 2005; Saccheri et al., 2008), species interaction (Kurz et al., 2008, Harrington et al., 1999) and ecosystem productivity (Lenihan et al., 2008; O'Reilly et al., 2003). In response to the abiotic modifiction inducted from antropic activity (particularly uncontrolled deforestation and greenhouse gas emissions), studies on the best species to be used in Short–Rotation coppice Cultures (SRCs), as a source of carbon-neutral renewable energy, are desirables.

In this direction our objectives are:

- to look at the phenotypic and genetic variation available in the POP5 family in terms of aboveground biomass production and biometric traits, and to analyse the phenotypic plasticity shown by the family growth in different environments;

- to analyse the behaviour shown by this family in different experimental trials, in order to evaluate the role played by age root sistem, climate conditions and silvicoltural management on biomass production;

- to evaluate the differences among model biomass estimation and to determine wheter the use of predictive equations could be standardized among contrasting environments;

- to propose a specific bud flush scoring system for *P. nigra* to be used as a landmark protocol to measure all aspects of the spring bud flush process dissecting its components at phenotypical and genetical level.

- to analyse the behaviour shown by the POP5 family in different growing seasons, in order to evaluate the role played by the temperature on the bud flush dynamics.

- to analyse the phenotypic plasticity of the bud flush process in two different growing seasons, a short-term option for adaptation (global change) that remains largely uncharacterized for phenology traits.

- to investigate the genetic architecture at the basis of biomass production and bud flush variance in *P. nigra*, to map QTLs (Quantitative Trait Loci) associated with these traits.

In perspective this work is aimed to contribute to the identification of one or more traits relevant as selection criteria and to provide informations on genomic regions involved in the expression of quantitative traits such as aboveground biomass production and bud flush phenology .

3. MATERIAL AND METHODS

3.1. Plant material

The full-sib family of *Populus nigra* L. (POP5) examined in this study consisted in 162 full-sib F_1. They were derived from a controlled intra-specific cross between two *P. nigra* genotypes with divergent phenotypes selected in natural Italian populations.

The female parent "58-861" originated from Northern Italy (45°09′ N, 7°01′ E), near the Dora Riparia river and close to the Italian Alps, at 597 m of altitude above sea level. It is adapted to the cool and moist conditions of the area, and may be considered "drought sensitive" (Cocozza et al., 2010). The male parent "Poli" was collected in Southern Italy (40°09′ N, 16°41′ E), near the mouth of the Sinni river close to the Ionio Sea, at 7 m of altitude above sea level. It is adapted to the dry and hot climatic conditions of the area and may be considered "drought-tolerant" (Gaudet et al., 2008). The parents originated from contrasting environments and had highly divergent phenotypes (Regier et al., 2009), to maximize phenological and biometric traits variability (Fig. 3.1).

Figure 3.1: Morphological differences between female "58-861" (on the left) and male "Poli" (on the right), parents of the *P. nigra* intra-specific cross.

The female parent has less branches and bigger leaves compared to the male parent as adaptive response to colder and wet environment conditions of the native site of origin. The cross was

realized in 2001 by Dr. Maurizio Sabatti at the Department for Innovation in Biological, Agro-food and Forest systems (DIBAF). The *P. nigra* F_1 progeny (POP5) is preserved by DIBAF in the experimental farm "N. Lupori" of the University of Tuscia at Viterbo, Italy.

3.2. Experimental plantations

The genetic material was replicated in different years and sites in northern and central Italy. Two experimental plantations were located in Lazio region and established during April 2003 in Montelibretti (MT) and April 2008 in Viterbo (VT) while other two experimental fields were planted in north-western Italy, in Piedmont region, during April 2003 in Cavallermaggiore (CV) and April 2008 in Savigliano (SAV). Hardwood cuttings (25 cm) were planted using a randomized complete blocks design with six blocks for CV, SAV and MT, and five blocks for VT. The planting distance of 2 m × 0.75 m was set up in CV, VT and MT. A larger spacing was used in SAV with a planting distance of 2.2 m × 0.8 m.

Figure 3.1: Preparation of two experimental plantations established during April 2003 in Cavallermaggiore (on the left) and April 2008 in Viterbo (on the right).

One ramet of each F_1 genotype and of the parents was randomly planted in each block. To minimize border effect two border rows of the *P. nigra* genotype "Jean Pourtet" were planted all around the plantation (Zavitkovski, 1981; van Hecke et al., 1995). The four plantations were irrigated, weeded and treated with pesticides and fungicides as needed during the trial period. CV and MT were coppiced in February 2005, and the latter site was removed in the same year. SAV plantation was cut down in February 2009, at the end of the first growing season, while VT was coppiced a first time in February 2009 and a second time in March 2011 (Tab. 3.1). After each coppicing all the experimental plantations were single stem grown removing the extra shoots on each stool. This practice was completed within mid-June to allow an unbiased growth of the shoot for phenological and biomass sampling.

Table 3.1: Summary table of silvicultural management of trial plantations.

Sites	Year of plantation	Year of coppicing
CV	2003	**no coppicing** at the end of the first growing season
MT	2003	**no coppicing** at the end of the first growing season
SAV	2008	**coppicing** at the end of the first (2009) growing season
VT	2008	**coppicing** at the end of the first (2009) and the third (2011) growing season

3.3. Sites description

The field trials were located in central (VT 42°25’ N - 12°05’ E; MT 42°8' N - 12°44' E) and northern Italy (CV; 44°42’ N - 07°41’ E; SAV; 44°36’ N - 07°37’ E;) with an elevation of 310 m, 148 m, 285 m and 321 m above sea level, respectively. Meteorological characteristics related to years of this study are shown for each site in Fig. 3.3. Climate data for Viterbo were provided by Department of Agriculture, Forests, Nature and Energy (DAFNE).

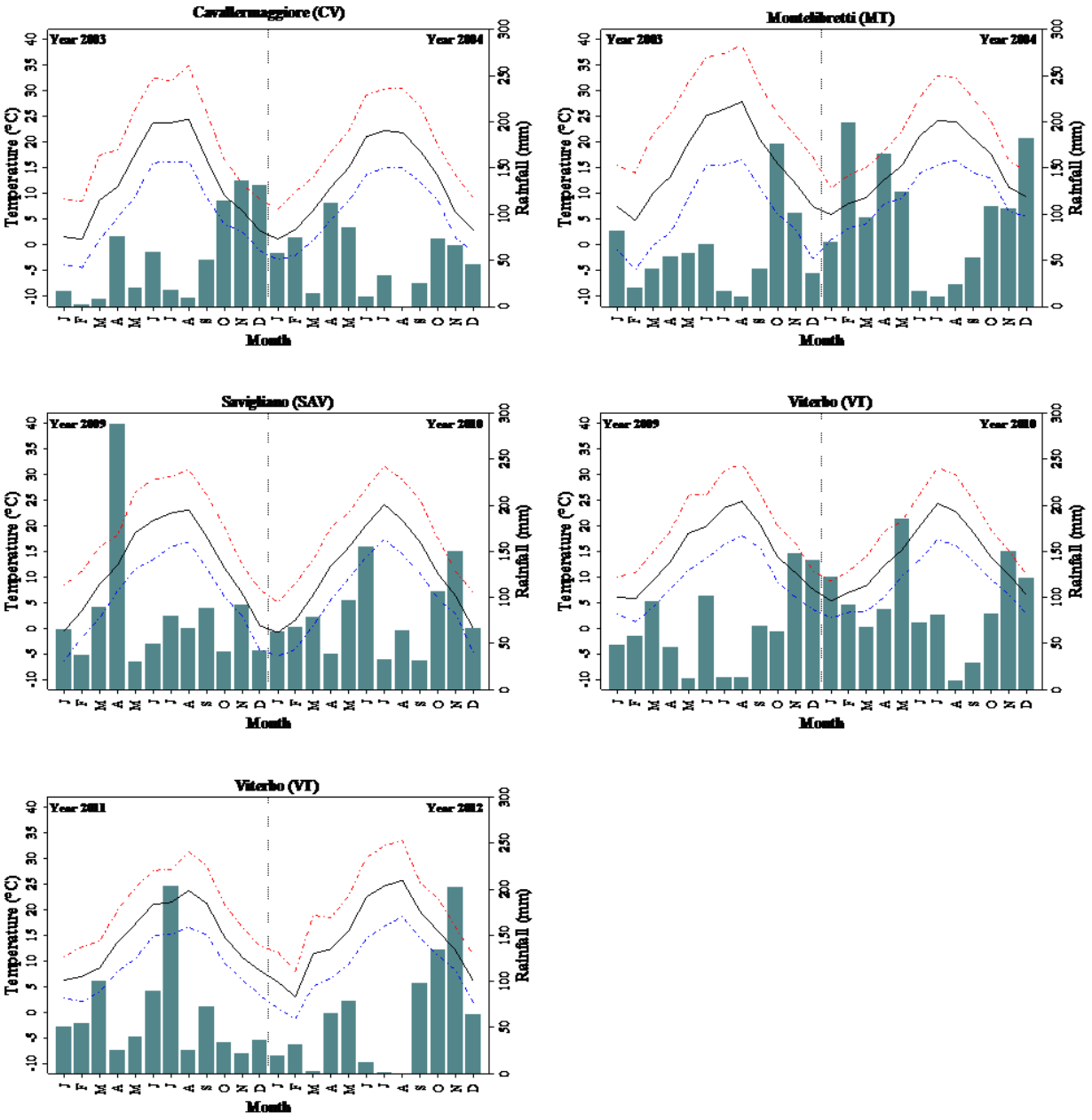

Figure 3.3: Meteorological characteristics observed in the different sites and years on the basis of the rotation (two years) of the poplar plantation. Monthly rainfalls (dark green bars) are plotted against right vertical axis. Monthly mean temperatures (solid black line), minimum temperatures (dashed blue line) and maximum temperatures (dashed red line) are plotted against left vertical axis.

Table 3.2: Seasonal and annual mean temperatures and cumulative rainfall observed in the different sites during the study years.

		Mean temperature (°C)					Cumulative rainfall (mm)				
Site	**Year**	**Winter**	**Spring**	**Summer**	**Fall**	**Annual**	**Winter**	**Spring**	**Summer**	**Fall**	**Annual**
CAV	2003	3.8	17.6	21.6	6.4	12.4	25.6	154.6	75.6	380.8	636.6
	2004	3.6	15.9	20.9	7.6	12.0	145.4	206.4	58.8	184.6	595.2
MT	2003	7.4	19.5	25.0	11.8	15.9	141.2	59.5	22.7	103.7	327.1
	2004	7.8	16.5	23.0	12.8	15.0	365.8	305.4	85.1	395.0	1151.3
SAV	2009	3.8	17.3	21.2	6.3	12.2	191.6	366.4	235.0	175.0	968.0
	2010	2.4	16.1	20.6	5.6	11.2	208.0	290.0	126.8	323.0	947.8
VT	2009	7.0	17.2	22.9	10.8	14.5	201.0	157.6	93.4	350.6	802.6
	2010	6.8	15.8	22.0	10.3	13.7	280.4	345.4	119.8	352.4	1098.0
	2011	7.3	17.3	22.1	11.2	14.5	205.0	154.6	300.0	90.9	750.5
	2012	6.8	16.9	23.4	11.3	14.6	51.4	155.0	97.8	398.4	702.6

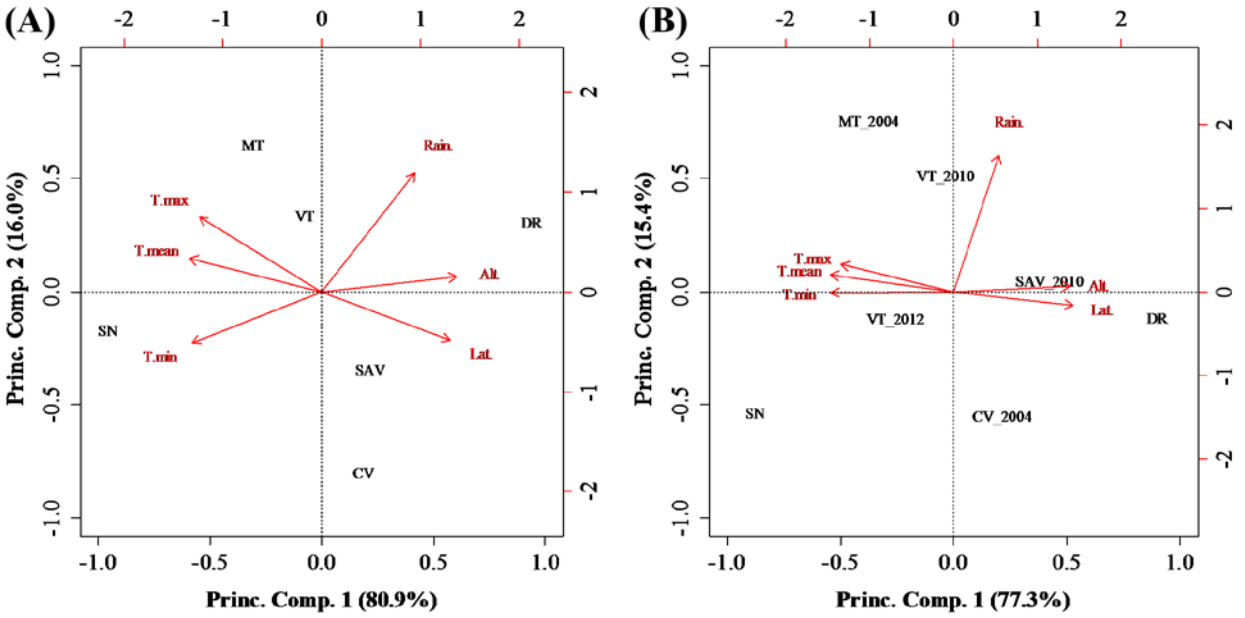

Figure 3.4: Climatic and geographic characterization of the sites using PCA (Principal Component Analysis). (A) Historical series of meteorological data. Annual mean, minimum and maximum temperatures (T.mean, T.min, T.max), annual rainfall (Rain.), altitude (Alt.) and latitude (Lat.) are the uncorrelated variables (red labels). Viterbo (VT), Savigliano (SAV), Montelibretti (MT), Cavallermaggiore (CV), Sinni (SN) and Dora Riparia (DR) river, are the factors (black labels). (B) Climatic data recorded during the experimental periods.

In the northern sites, characterized by greater temperature seasonality during the fall and the winter, mean temperatures during the year were always colder than in central Italy (Tab 3.2). Rainfall annual accumulation was in line with the time series for VT (728 mm) (Italian Air Force Weather Service, http://www.meteoam.it/) and CAV (569 mm) (Marron et al., 2010) while for MT it was observed a very low precipitation in 2003 (327 mm vs. 939 mm) (Meteo System s.r.l., http://www.meteosystem.com).

Parents provenance sites are strongly in contrast for temperatures and photoperiod (mainly related to the latitude of origin), and the variance in the mean annual rainfall regimes are less remarkable (fig. 3.4). The four experimental sites show significant differences only between northern (CV and SAV) and southern sites (MT and VT) for rainfall. Taking in consideration only the summer period, in MT and VT sites were observed very low values of rainfall, compare to precipitation occurred in fall and winter. This is typical of Mediterranean climate characterized by arid summer with few precipitations. Differently, for CV and SAV were observed greater variance of rainfall distribution among seasons, suggesting a more oceanic climate. However, the near Alps Mountain exercise a large effect on rainfall regime, because they intercept the precipitation from the oceanic western zones. For CV and MT, the temperatures observed during the spring and the summer of 2003 resulted to be higher than temperatures observed in the following year 2004.
The soil at CV and SAV was an alluvial soil with sandy-loam texture while it was sandy silt in VT and MT (Sabatti, personal communication).

3.4. Data sampling and database construction

In this section are describe the protocols used to collect phenological and biomass data in fields, and the organization of database with the collected and derived traits.

3.4.1. Aboveground biomass data collection

To select a sample of genotypes representative of the genetic variation of POP5 family, circumference and height of stem were collected in the experimental plantations. At the end of first growing season of each biennial rotation (winter 2003-2004 in CV and MT, winter 2009-2010 in SAV, winter 2009-2010 and 2011-2012 in VT), height (Height1) of all shoots was measured to the nearest cm, using a graduated height pole. In the same period, number of sylleptic branches of each shoot (Syll1) was collected in CV and MT. At the end of second growing season of each biennial rotation (December 2004 in CV and MT, December 2010 in SAV, December 2010 and December 2012 in VT), circumference (Circum2) was measured, to the nearest mm at 1 m above the ground using a graduated tape. At the same time, height (Height2) of all shoots was measured for the whole family in CV and MT.

Table 3.2: Time table of biomass data sampling. “Biomass” indicates fresh mass of branches, stem and whole tree.

	1st ROTATION						2nd ROTATION		
Site	**December 2003**	**December 2004**	**January 2005**	**December 2009**	**December 2010**	**February 2011**	**December 2011**	**December 2012**	**February 2013**
CV	Height1 Syll1	Height2 Circum2	Biomass						
MT	Height1 Syll1	Height2 Circum2	Biomass						
SAV				Height1	Height2 Circum2	Biomass			
VT				Height1	Height2 Circum2	Biomass	Height1	Height2 Circum2	Biomass

For each site, a sample of 20 genotypes representative of the genetic variation in growth and sylleptic ramification was selected for the development of yield equations using sampling optimization (n = 10 000) with the following quality criterion (QC): (Dillen et al., 2007; Marron et al., 2010a):

$$QC = \left[\mathrm{var}\left(\mathrm{Circum2}_{sample}\right) - \mathrm{var}\left(\mathrm{Circum2}_{family}\right)\right]^2 + \left[\mathrm{var}\left(\mathrm{Height1}_{sample}\right) - \mathrm{var}\left(\mathrm{Height1}_{family}\right)\right]^2 + \left[\mathrm{cov}\left(\mathrm{Circum2} - \mathrm{Height1}_{sample}\right) - \mathrm{cov}\left(\mathrm{Circum2} - \mathrm{Height1}_{family}\right)\right]^2$$

where:

var: variance,

cov: covariance,

$_{sample}$: tested sample mean value,

$_{family}$: family mean value.

Three plants per genotype of the 20 selected genotypes and parents showing the closest performance to the family mean were harvested in the four sites (a total number of 66 trees collected for each site).

Aboveground biomass sampling was performed in MT and CV during January 2005 , at the end of the first biennial rotation (R2-S2). In February 2011, biomass data were collected in SAV at the end of the first rotation (R3-S2). Biomass sampling was made in VT during February 2011 and during February 2013, at the end of the first (R3-S2) and second (R5-S2) biennial rotation. Selected clones were cut at ground level, and total fresh biomass (TotalFM) and stem fresh biomass (StemFM) were

determined separately, using a digital field scale with an accuracy of 1 g (Fig. 3.5). Branch fresh biomass (BranchFM) was estimated as the difference between TotalFM and StemFM. Two wood samples per plant were randomly collected on the portion of stem developed during the first and the second growing season). Two random samples of branches were also obtained from a mix of sylleptic and proleptic branches taken at different height of the stem (Fig. 3.5). These samples of fresh biomass of stem (S_1FM and S_2FM) and branches (B_1FM and B_2FM), were weighed in the laboratory with a digital precision scale (accuracy of 0.01 g) (Fig. 3.5).

Figure 3.5: Digital field scale used for the measurement of total stem fresh weight (on the left), collection in the field of random samples of branches (in the middle) and digital precision scale used in the laboratory (on the right).

Using a drying oven, the samples were dried at 70 °C for an average period of 5 days. The anhydrous condition of the wood samples was established until there is no difference between any two consecutive measurements of the weight of samples. Dry biomass weight of the stem (S_1DM, S_2DM) and branch (B_1DM, B_2DM) samples was obtained with the same digital precision scale. Before the harvesting operations in CV and MT, the height (Height2) was measured on all the trees of the POP5 family. Differently, in SAV and VT, only the height of the selected genotypes was collected.

3.4.2. Development of the estimation model

Fresh and dry stem biomass (SFM and SDM) and fresh and dry branch biomass (BFM and BDM) mean values were used for the determination of the stem dry/fresh ratio (StemD/F) and branch dry/fresh ratio (BranchD/F):

$$\mathrm{StemD/F} = \mathrm{SDM/SFM}$$
$$\mathrm{BranchD/F} = \mathrm{BDM/BFM}$$

Stem dry mass (StemDM), branch dry mass (BranchDM) and total dry mass (TotalDM) were calculated for all the sampled trees with the following equations:

$$\mathrm{StemDM} = \mathrm{StemFM} \times \mathrm{StemD/F}$$
$$\mathrm{BranchDM} = \mathrm{BranchFM} \times \mathrm{BranchD/F}$$
$$\mathrm{TotalDM} = \mathrm{StemDM} + \mathrm{BranchDM}$$

Statistical R software (version 2.14.2. A Language and environment Copyright, 2007) was used to develop and to evaluate biomass estimation models. Multiple regression linear analysis was performed in order to calculate StemDM, BranchDM and TotalDM on the whole family, using Circum2, Height2 and Syll1 as scaling factors or predictors (Dillen et al. 2007) using the following equation:

$$\mathrm{DM} = \beta_0 + \beta_1 \times \mathrm{Circum2} + \beta_2 \times \mathrm{Height2} + \beta_3 \times \mathrm{Syll1}^*$$

where:

DM:	dry mass (StemDM, BranchDM, TotalDM),
Circum2, Height2, Syll1:	predictors (independent variables),
β_0, β_1, β_2, β_3:	parameters estimated using the method of least square,

*: data present only for the sites of CV and MT.

In order to get a normal distribution of residuals according to linear regression assumptions, dependent variables (e.g. DM) were transformed when required (Sokal and Rohlf, 1995). The Box-Cox procedure was performed to determine the best λ value for each transformation (Box and Cox, 1964).

A backward analysis approach was used to fit the best regression model (with the highest coefficient of determination R^2) and to obtain the minimum number of significant predictors (principle of parsimony). Predictors were tested for the full multiple model (including all the possible predictors) and removed from the equation if *P*-value was non-significant ($P > 0.05$). After each removal, the test was repeated until to keep only significant predictors in the model. Significant β parameters were used for the assessment of biomass for each genotype.

With the aim to estimate height of each clone of the plantation for SAV and VT sites, collected Height2 and Circum2 were used to create a height-diameter curve (Fig. 3.6).

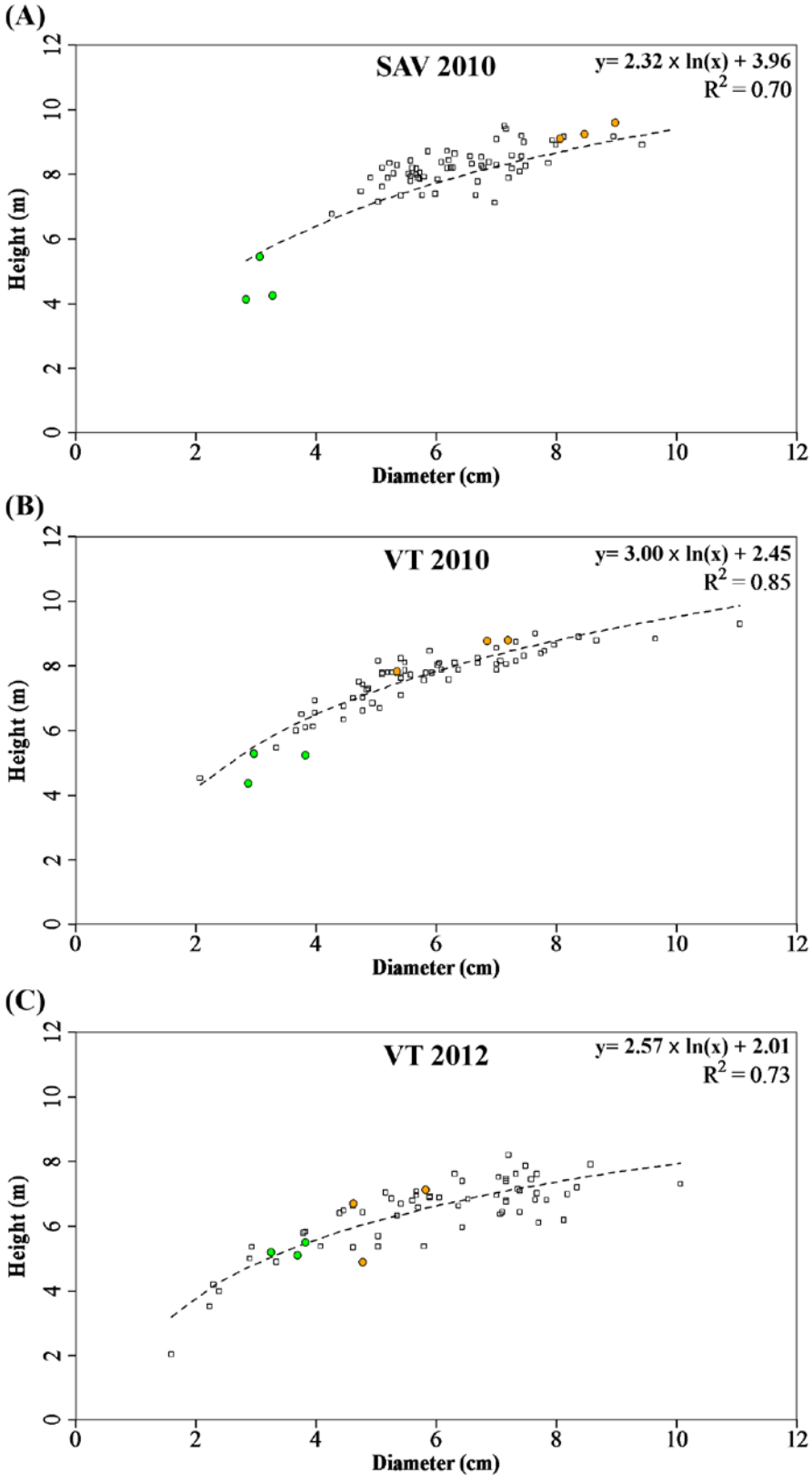

Figure 3.6: Regression diameter-height for the sites of SAV 2010 (A), VT 2010 (B) and VT 2012 (C). A semi-log regression was used. R^2 is the coefficient of determination of the regressions (dashed line). Selected offspring genotypes (black open square), male parent "Poli" (green circles) and female parent "58-861" (orange circles) are shown.

Average bias (B) and standard error of estimate (SEE) were calculated for all the regressions to give an estimate of the model accuracy. B is defined as (Dillen et al., 2007):

$$B = \frac{\sum_{i=1}^{n} y_i - \hat{y}_i}{n}$$

where:

y_i: collected value of i^{th} genotype,

$\hat{y}_i$: estimated value by the regression of i^{th} genotype,

n: number of observation.

Each calculated value of B is expressed as percentage ($B_{\%}$) in respect of the collected values in the field to compare models obtained for different environments. A positive number of B stands for an underestimation of the model in relation to sampled data, while a negative number means an overestimation. SEE represents the square root of the mean square residual and is defined as (Zabek and Prescott, 2006):

$$SEE = \sqrt{\frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{n - m - 1}}$$

where:

m: number of independent variables (predictors) in the model.

SEE is a measure of the data spread around the regression line. Low value of SEE indicates a better fit to the data (Zabek and Prescott, 2006). Like for B, the SEE is expressed in percentage ($SEE_{\%}$), to compare results obtained for different sites.

Realized aboveground wood productivity (AWP_{real}) expresses the mean yield performance (i.e. ton) per unit of time (i.e. year), per unit of surface (i.e. hectare). It was calculated in agreement with equation proposed by Marron and coworkers (2010a):

$$AWP_{real} = \frac{\left(\frac{\mu_{TotalDM}}{10^6} \times n_{hectare} \times S\right)}{t}$$

where:

AWP_{real} : Realized aboveground wood productivity (t ha^{-1} $year^{-1}$)

$\mu_{TotalDM}$: family mean values of TotalDM (g),

$n_{hectare}$: number of trees per hectare

S: survival rate (%) in the plantation,

t: number of growing season (year),

10^6: conversion factor from grams to tons.

Realized aboveground wood productivity is a useful parameter to compare biomass yields among the different environments here studied and with values presents in the bibliography.

3.4.3. Bud flush data collection

Bud flush data collection was performed according to a scoring system developed by Castellani et al. (1967), to optimize chemical treatments in hybrid poplar nursery (Fig. 3.7).

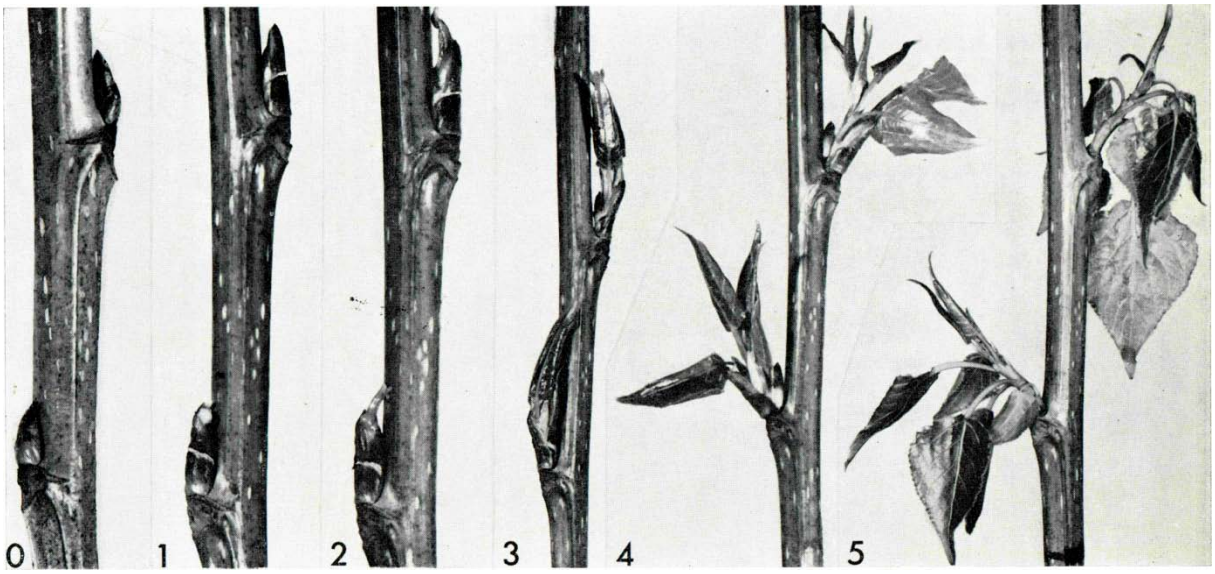

Figure 3.7: Original scoring system for the characterization of the bud flush process (Castellani et al., 1967)

This protocol was used to assign at each phenological stage a different score, proceeding from dormant buds (*"fase 0"*) to the elongation of petiole and to the complete stretching of leaves (*"fase 5"*). In some traditional studies of bud flush phenology, the equivalent of phase 4 (*"fase 4"*), and sometime phase 2 (*"fase 2"*), correspond to the most utilized stages for process characterization (Pellis et al., 2004; Luquez et al. 2008; Howe et al., 2000; Bradshaw and Stettler 1995).
The proposed protocol used in this study was developed on the basis of the previous system, and these are its main points:

- the protocol is suited to control the 3-4 apical buds of the main stem in *Populus nigra* L,

- all high resolution pictures were taken on individuals of *Populus nigra* L. during the phenological sampling,
- each phase was renamed from 0.5 to 2.5, creating correspondence with the bud set protocol published by Rohde and coworkers in 2011 , in order to propose a unified phenology protocol for poplar,
- in order to focus the attention on the leaves formation, the duration of leaf initiation, called *subprocess1*, was identified from bf_date1 (*ex fase 2*) to bf_date2 (*ex fase 4*). The bf_date1.5 (*ex fase 3*) was considered taking into account leaves elongation, with their length longer than bud scales.
- The whole time process was dissected in duration of each stage, in order to analyze the speed of the process in respect to temperature variations.

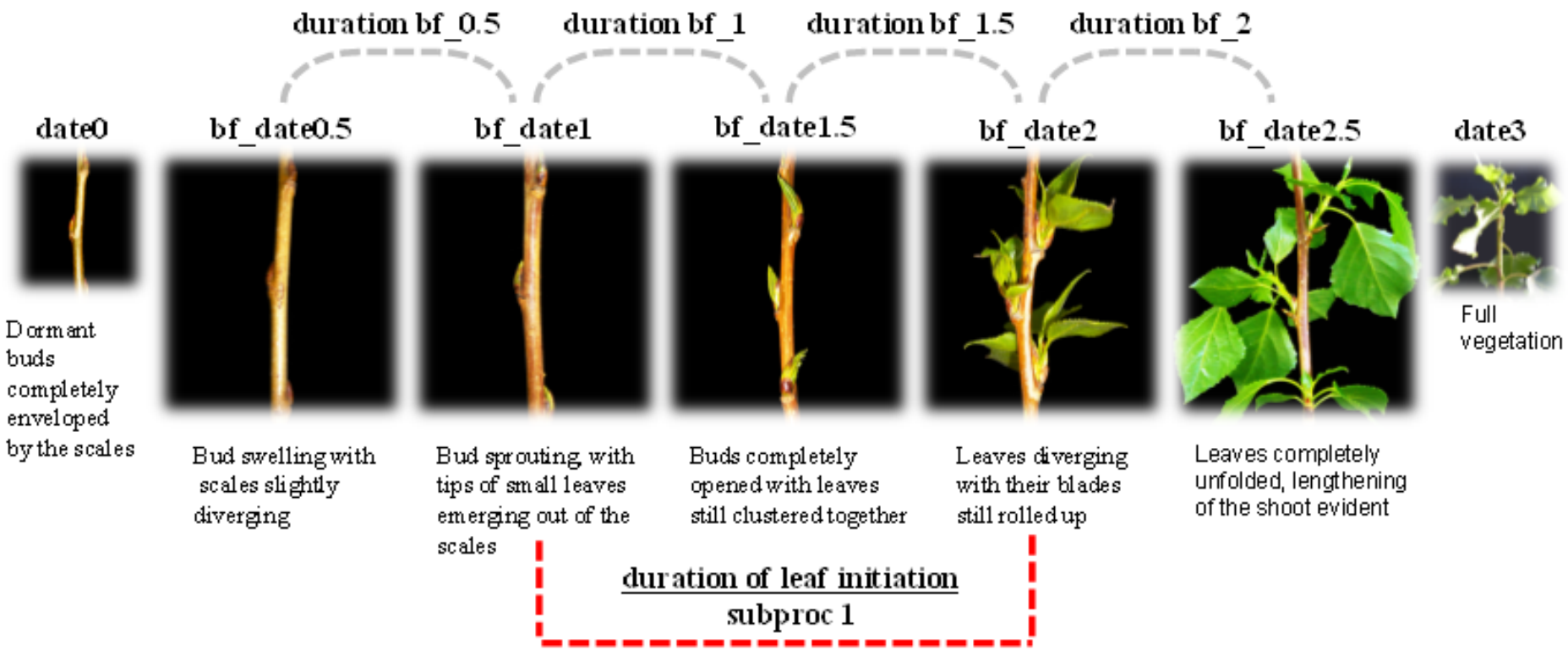

Figure 3.8: Scoring system for bud blush process developed and used in this study.

The protocol allows to improve the phenotypic resolution of the distinctive stages observed during the breakup of the terminal buds in spring and to evaluate the stage duration of leaves development as separate character. For this protocol (Fig. 3.8) five representative phases (excluding bf_date3, which represents the full vegetation), plus one stage not strictly linked to leaves formation (bf_date0, which represent the dormant bud), were defined to characterize the bud flush dynamics. The protocol covers the bud flush process from the bf_date0.5, that represents the perception of threshold temperature and the onset of the phenological process, to bf_date2.5, that indicates the total stretching of leaves (Fig. 3.8).

Phenological measurements were collected in VT, during spring 2010 and spring 2012. Data were collected at the beginning of the second growing season, after the first biennial coppice rotation, with root and shoot ages of 2 and 1 years (R2-S1) respectively, and after the second biennial rotation cycles, with root and shoot ages of 4 and 1 years (R4-S1), respectively. Bud flush was scored every two-three days, from March 22 (*Day of the year* – DOY = 81) up to April 21 (DOY = 111) in 2010 and from March 7 (DOY = 67) up to April 6 in 2012 (DOY = 97).

Other available data by former works were used to make comparisons. In 2010 for the site of SAV (R2-S1), bud flush stages were scored for 6 genotypes and “Poli” and “58-861” parents, whereas in 2004, for MT and CV sites (R1-S1), bf_date1.5 was scored for all the full-sib family (Tab. 3.4). On each day of observation the stage of the apical buds was recorded by giving each tree a score according to the correspondence with the phenological protocol. All the trees that showed some stressful condition, likely to influence the natural phenological process or with damage to the apical meristems, were excluded from all measures.

Table 3.4: Time table of bud flush data sampling.

	1st ROTATION		2nd ROTATION
Site	**March-April 2004**	**March-April 2010**	**March-April 2012**
CV	Bud flush		
MT	Bud flush		
SAV		Bud flush	
VT		Bud flush	Bud flush

3.4.4. Bud flush data management and fitting

Genotypes with less than three replicates were excluded from further data analysis. Recorded data in DOY were used to define complementary temperature traits. The concept is based on a linear relationship between temperature and developmental rate of the plant (Baker et al., 1984).

In order to estimate stages that were not observed in the field, data were smoothed using a local polynomial regression of degree 2 (LOESS) (Cleveland et al., 1992) (Fig. 3.9). The sampling dates were used as predictors, in order to retrieve a data for missing scores in respect to the period of observation.

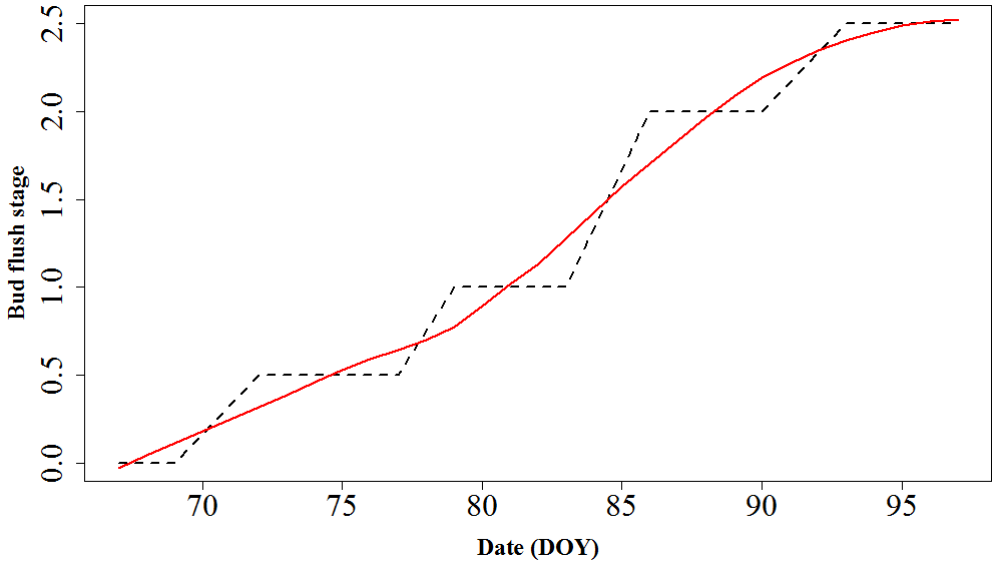

Figure 3.9: The result of fitting procedure concerning a generic individual. The figure shows the raw data trend (broken dashed black line) and the locally weighted scatter plot smoothing (solid red line). In this example it is evident the lack of data for stage 1.5 and its estimation.

3.4.5. Temperature accumulations

To investigate the relevancy of temperature fluctuations on bud flush dynamics, a heat unit model approach was employed. Daily minimum temperatures below 5°C were cumulated from November 1st (CMT_5), in order to analyze the role of low temperatures on the dynamics of bud flush. Daily mean temperatures sum above 0°C (CAT_0) and daily average temperatures above 10°C (CAT_{10}) were cumulated from November 1st, in order to get values to be used for bibliography comparisons. November 1st was chosen as starting date of temperatures accumulation, taking into account that previous development stage, corresponding to the apical bud fully closed (Fabbrini et al. 2012), was completed.

Growing degree days and base temperature notions (Ruml et al., 2010; Yang et al., 1995; Arnold 1959; Gilmore and Rogers 1958) were used to evaluate heat and cold accumulation for all bud flush stages and their durations.

Heat sum above 0°C and 10°C were calculated using the following equation (Ruml et al., 2010):

$$CAT_{i,m} = \sum_{k=1}^{n} CAT_k$$

with,

$$CAT_k = t_k - t_b$$

where:

$CAT_{i,m}$: Cumulative average temperature above t_b of the i^{th} tree, for the m^{th} stage,

t_k: daily mean temperature of the k^{th} day,

t_b : imposed base temperature of 0°C (CAT_0) or 10°C (CAT_{10}),

n: total days of accumulation since starting date.

The daily negative CAT_k values, were set equal to zero.

Cold sum below 5°C were calculated in conformity with previous equation:

$$CMT_{i,m} = \sum_{k=1}^{n} CMT_k$$

with,

$$CMT_k = t_b - t_k$$

where:

$CMT_{i,m}$: Cumulative minimum temperature below t_b of the i^{th} tree, for the m^{th} stage,

t_k: daily minimum temperature of the k^{th} day,

t_b : imposed base temperature of 5°C (CMT_5),

n: total days of accumulation since starting date.

The daily positive CMT_k values, were set equal to zero.

3.5. Statistical analysis

3.5.1. Comparison among regression models

ANalysis of COVAriance (ANCOVA) was performed to determine whether the use of more estimation equations could be standardized among sites or years, that is to use one equation for more environments. Analysis of covariance combines elements from regression and analysis of variance (ANOVA). The purpose of ANCOVA is to test whether the mean of dependent variable Y is different for distinct factor levels, taking the covariates X into account (Sokal and Rohlf, 1995). There is at least one continuous explanatory variable (covariate) and at least one categorical explanatory variable (factor). A generic procedure works like this (Crawley, 2005) (Fig. 3.10):

- fit two or more linear regressions of Y against X (one for each level of the factor),
- estimate different slopes and intercepts for each level,
- use the model simplification (deletion tests) to eliminate unnecessary parameters.

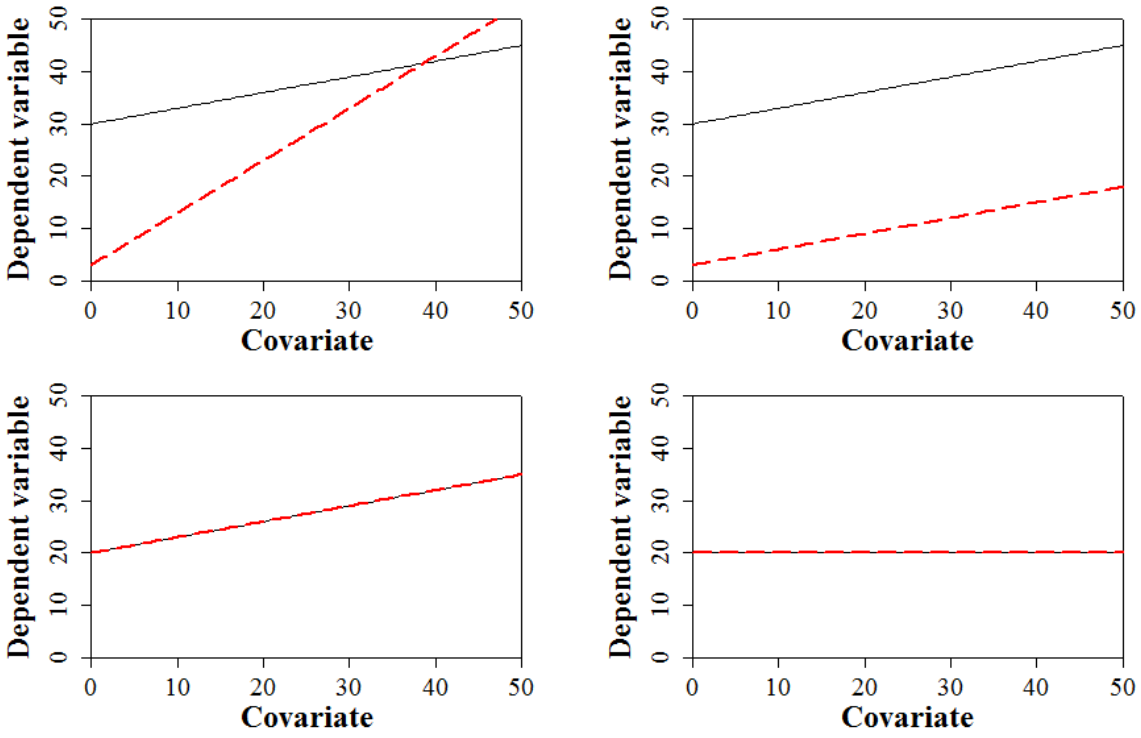

Figure 3.10: General scheme of ANCOVA. Different slopes and intercepts show significant different effects of covariate, factor and their interaction on the dependent variable (upper left). A not-significant effect of interaction between factor and covariate, allows a simplification model with imposition of the same slope value (upper right). Subsequently, a not-significant effect of factor levels on the response, allows mathematical restriction whit the same slope and intercept values (bottom left). In the limit, neither the covariate nor the factor have any significant effect on the response (bottom right).

In our case, more estimation equations were compared to check the opportunity to use one mathematical formula to estimate the biomass yield for different sites and years. The following ANCOVA model was performed with Circum2, Height2 and Syll1 as covariates, and sites (VT, MT, CV, SAV) and years (2010, 2012) as levels of the factor (Dillen et al., 2007):

$$\mathrm{DM} = \beta_0 + \beta_1 \times \mathrm{Circum2} + \beta_2 \times \mathrm{Height2} + \beta_3 \times \mathrm{Syll1} + \beta_4 \times \mathrm{Factor} + \beta_5 \times \mathrm{Circum2.Factor} + \beta_6 \times \mathrm{Height2.Factor} + \beta_7 \times \mathrm{Syll1.Factor}$$

where:

$\mathrm{Circum2.Factor}$, $\mathrm{Height2.Factor}$, $\mathrm{Syll1.Factor}$: interaction components of the variance.

Due to different age of the root systems, ANCOVA was performed between sites with the same age (CV 2004 vs. MT 2004 and SAV 2010 vs. VT 2010). Having two biomass samplings for different years in VT site (2010 and 2012), comparison between two growing seasons was performed (VT 2010 vs. VT 2012).

3.5.2. Analysis of variance within environment

Individual data of phenology and biomass production were analyzed with the inferential statistic procedure of ANOVA (ANalysis Of VAriance). In order to describe the effects of different sources of variance within each environment (site and year), the following linear additive model was performed:

$$Y_{ij} = \mu + B_i + G_j + \varepsilon_{ij}$$

where:

Y_{ij}: observation for the j^{th} genotype in the i^{th} block,

μ: general mean,

B_i: effect of the i^{th} block (fixed),

G_j: effect of the j^{th} genotype (random),

ε_{ij}: residual error.

Block effect (B_i) was estimated as the difference between the mean of i^{th} block and the general mean (μ) of the whole population (Dillen et al., 2009). Differences among genotypes and among blocks were considered significant when the *P*-value of ANOVA was ≤ 0.05. When the within site block effect was significant, all individual values were adjusted as:

$$Y'_{ij} = Y_{ij} - B_i$$

where:

Y'_{ij}: are the adjusted individual values.

To respect the ANOVA assumptions (homoscedasticity of variance and normality distribution of residual errors), Box-Cox procedure (Box and Cox, 1964) was performed on the additive model, to find the best value of λ for data transformation (Fig. 3.11).

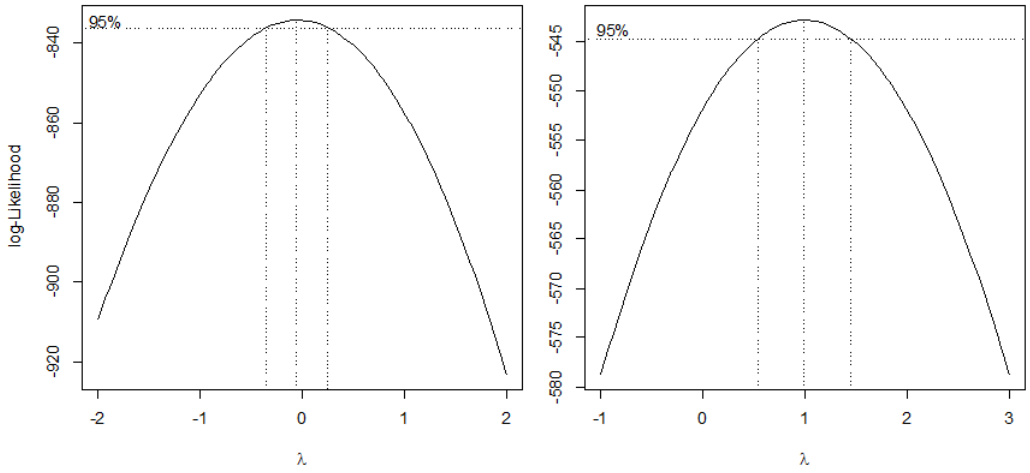

Figure 3.11: Box-Cox procedure application. In this example, the test suggests a transformation with natural logarithm (on the left, λ=0) to obtain a normal distribution of residuals (on the right, λ=1).

3.5.3. Analysis of variance between environments and genotype plasticity

In order to describe different family performances observed between two different environments (sites or years), genotype by environment interaction was taken into account. Genotype by Environment interaction (G×E) is a lack of consistency in the relative performance of genotypes when they are grown in different environments. Statistically, G×E is a two-way interaction in an ANOVA that occurs when true differences between genotypes are not constant in all environments in which they are planted (White et al., 2007). A linear additive model is insufficient to explain the

observed variation, so to explain interaction another term ($G \times E_{jk}$) must be added (Dillen et al., 2009). In the present work, having samples from more sites and more years, in addition to a G×E analysis between sites, an evaluation of interaction between years was made using the following model:

$$Y'_{ijk} = \mu + G_j + E_k + G \times E_{jk} + \varepsilon_{ijk}$$

where:

Y'_{ijk}: individual adjusted values for within site block effects,

μ: general mean between sites,

E_k: effect of the k^{th} site or year (random),

G_j: effect of the j^{th} genotype (random),

$G \times E_{jk}$: genotype by site (or year) interaction effect (random),

ε_{ijk}: residual error.

To describe the relative importance of each random effects in phenotypic variation (σ^2_P), REstricted Maximum Likelihood (REML) method was used to estimate variance components, that can be related to: the environment (σ^2_E), genetic (σ^2_G), genetic by environment interaction ($\sigma^2_{G\times E}$) and residual component (σ^2_ε).

In case of significant G×E interaction, the non-parametric Spearman rank correlation coefficient (ρ) was calculated to study relationships between different rankings of the same variable (in this case, the same trait in different environments). Different genotypic mean performances were checked with (Soliani, 2005):

$$\rho = 1 - \frac{6 \times \sum_{i=1}^{N} d^2_{R_i}}{N^3 - N}$$

where:

ρ: Spearman rank correlation coefficient,

$d^2_{R_i}$: difference between each rank of the corresponding values of x_1 and x_2,

N: number of observation pairs.

A ρ value of -1 or +1 suggests a complete dependence between variables (the same rank position of genotypes), inverse or direct correlation respectively. A ρ value equal to 0 indicates a total independence between variables, representing a random changes in genotype ranks. However, to confirm real random changes in ranks, statistical significance of the test must to be not significant (P-value≥0.05).

In order to dissect the contribution to G×E interaction and understand how many genotypes and how they contribute to this source of phenotypic variability, Wricke's ecovalence was performed. The ecovalence (W_i^2), or stability of the i^{th} genotype, is its interaction with the environments (site or year), squared and summed across environments, and expressed as (Wricke, 1962; Annichiarico, 2002):

$$W_i^2 = \sum_{i=1}^{n} \left(R_{ij} - m_i - m_j + m\right)^2$$

where:

W_i^2: relative ecovalence of i^{th} genotype,

R_{ij}: mean performance of i^{th} genotype in the j^{th} environment,

m_i: genotype mean across environments,

m_j: environment mean,

m: overall mean.

Genotypes with a low W_i^2 value have smaller deviations from the mean across environments and are thus more stable (Annichiarico, 2002). It's moreover possible to evaluate the relative contribution of each site analyzed in the interaction by the relative ecovalence of the site as (Wricke,1962; Brancourt-Hulmel et al., 1997):

$$W_j^2 = \frac{\sum_{j=1}^{n} (G \times E)_{ji}^{\ 2}}{\sum_{ji=1}^{n} (G \times E)_{ji}^{\ 2}}$$

where:

W_i^2: relative ecovalence for a given environment j.

3.5.4. Phenotypic correlation between traits

Strength phenotypic dependence between two traits was estimated by Pearson's linear correlation coefficient:

$$r_{(xy)} = \frac{cov_{(xy)}}{\sigma_x \times \sigma_y}$$

where:

$cov_{(xy)}$: covariance of the two variables x and y,

σ_x, σ_y: standard deviation of the two variables x and y.

The Pearson's correlation coefficient is +1 in the case of a perfect increasing linear relationship,−1 in the case of a perfect decreasing relationship and 0 when the variables are completely independent. All values between the minimum and maximum indicating the degree of linear dependence between the two variables tested.

3.5.5. Genetic parameters

Broad-sense heritability was calculated for each trait at individual (H_i^2) and genotypic (H_g^2) level using (Monclus et al., 2012) variance components obtained with REML method with the following relationships:

$$H_i^2 = \frac{\sigma_G^2}{(\sigma_G^2 + \sigma_\varepsilon^2)}$$

where:

σ_G^2: genetic component of variance,

σ_ε^2: residual component of variance.

and,

$$H_g^2 = \frac{\sigma_G^2}{\left[\sigma_G^2 + \left(\sigma_\varepsilon^2 / n_j\right)\right]}$$

where:

$n_{j:}$ average number of replicates per genotype.

Heritability on genotypic basis (H_g^2) gives information about the precision of estimated genetic values when genotypic means were used as phenotypic predictors. Heritability on individual basis (H_i^2) can be considered as a reference value, easily comparable to literature (Monclus et al., 2012).
To compare the variance of different sources of variation (genetic and environmental), for traits with different means, Genetic Coefficient of Variation (CV_G) and Residual Coefficient of Variation (CV_ε) were calculated (Soliani, 2005):

$$CV_G = \frac{\sigma_G}{\mu} \times 100$$

and,

$$CV_\varepsilon = \frac{\sigma_\varepsilon}{\mu} \times 100$$

where:

σ_G: standard deviation of genetic variance,

σ_ε: standard deviation of residual variance,

μ: family mean.

3.5.6. Principal component analysis

To summarize the variability and the relationships between phenological traits, multivariate analyses using principal components analysis (PCA) were performed for each experimental location. The traits were standardized and orthogonal factors were successively established as linear combinations of these traits to maximize variability explained by these factors (Dillen et al., 2009). Results obtained from heritability calculation, linear correlations and PCA, were used to select the traits which account for most of the variance in the observed phenological process of bud flush and to describe genetic relationship between the different bud flush stages with the help of subsequent QTL analysis.

3.6. QTLs analysis

QTLs were determined using MultiQTL 2.5 software (http://www.multiqtl.com/; MultiQTL Ltd, Institute of Evolution, Haifa University, Haifa, Israel). The genetic linkage map for the full-sib family POP5, were constructed using 154 *P. nigra* genotypes (Gaudet et al., 2008). Genetic maps were drawn using MapMaker software version 3.0. QTLs analysis was performed with the same 154 genotypes, using the block-adjusted genotype means of selectedtraits. Only genotypes with at least three surviving clones were included in the analysis. A multi-environment analysis (i.e. the number of phenotypes is equal to number of genotypes multiplied by the number of environments), was performed for each trait of interest, with data collected in more trials.

Three different data sets were analyzed in order to give information from comparisons of more sites in the same years, CV 2004 vs. MT 2004 and SAV 2010 vs. VT 2010, and in the same site but different years, VT 2010 vs. VT 2012. This approach of analysis allows to check the presence of same QTLs in more conditions (time or location) (Tab. 3.5).

Table 3.5: Data sets used in multi-environment analysis for QTLs detection.

Environments	**Common conditions between trials**
CV 2004 vs. MT 2004	no coppicing at the end of the first growing season
SAV 2010 vs. VT 2010	coppicing at the end of the first growing season
VT 2010 vs. VT 2012	same location and silvicultural management

Single trait analysis was performed using a combination of interval mapping approach and multiple interval mapping (MIM). As first hypotheses, a single QTL per linkage group (LG) was presumed (H1:H0), assuming a single model for genome investigation. For all putative QTLs (LOD≥2), LOD value significance was tested with a series of permutation tests (1000 runs and p-value≤0.05). For chromosomes for which a single significant QTL was detected, permutation tests were run to compare the hypotheses of two linked QTL versus a single QTL per LG (H2:H1). When H2 versus H1 hypotheses was accepted (p-value≤0.05), permutation tests were run to compare the hypotheses H2 versus H0, to ensure that the two-linked QTL model fitted the data better than a single QTL model (Monclus et al., 2012). Moreover, all QTLs (single and two-linked) detected with single interval analysis, were tested with MIM. This more powerful statistic method allows confirming results obtained with previous procedure. Bootstrap analysis was performed to estimate the 95%

confidence intervals. Phenotypic explained variation (PVE) by all the QTLs for a same trait was estimated by MIM for each parental map separately.

In the last step, differences between additive genetic components at the different sites were tested by creating sub-models with the additive genetic components set as equal at all sites. Permutation tests (1,000 runs) were carried out to compare the sub-model to the original model where the additive genetic components were estimated separately for each site. Where models were seen to differ significantly, the genetic components of the mapped QTL were assumed to differ across sites.

4. RESULTS

4.1. Survival

Plant survival rate was estimated using the total number of individuals sampled during phenological measurements (i.e. during the spring), with respect to the total number of individual of whole plantation. Overall POP5 survival rate was 80.3% in CV 2004, 76.2% in MT 2004, 86.3% in SAV 2010, 88.9% in VT 2010 and 68.4% in VT 2012, with the lowest value of survival rate (47.3 %) for parents in VT 2012. An evident reduction occurred in VT between 2010 and 2012 (age roots of 2 and 4 years, respectively) (Tab. 4.1), due to different stumps sprouting ability in the years and to competition among genotypes. Beginning stage of bud flush (bf_date05) and circumference collected at the end of the second growing season (Circum2) were used to check the distribution of died plants in VT 2012 respect the performances of these clones observed in VT 2010. For Circum2, distribution of died clones was the same of the overall performance observed on VT 2010 (Fig. 4.1 B). The same behavior was observed for bf_date0.5, with a slight imbalance of mortality to the earliest genotypes (Fig. 4.1 A). Summarizing, these results reveal a random mortality of genotypes between years, indifferently to performances observed in 2010.

Table 4.1: Survival rate observed in the different environments for POP5 family.

Env.	Progeny		Poli		58-861	
	Survival	Mortality	Survival	Mortality	Survival	Mortality
CV 2004	81.3	18.7	83.3	16.7	83.3	16.7
MT 2004	77.2	22.8	66.6	33.4	83.3	16.7
SAV 2010	88.4	11.6	50	50	75	25
VT 2010	93.3	6.7	68.4	31.6	52.6	47.4
VT 2012	71.7	28.3	47.3	52.7	47.3	52.7

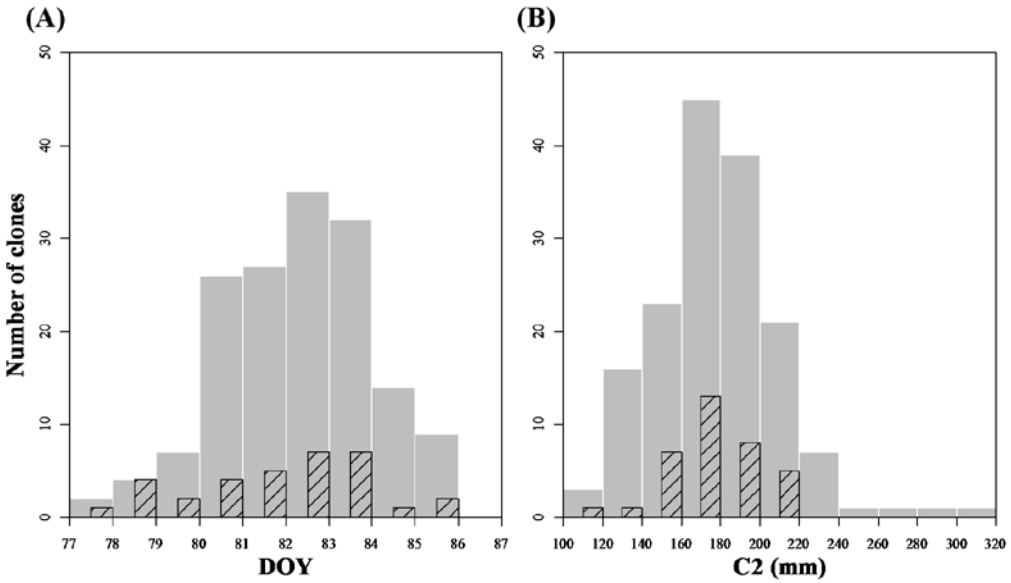

Figure 4.1: Mortality occurred in VT 2012. Number of clones died in VT 2012 (dashed bars) with respect to their performances observed in VT 2010 (grey bars). (A) Bud flush beginning (bf_date0.5) expressed in DOY, (B) circumference related to the end of the second growing season (Circum2).

4.2. Aboveground biomass

4.2.1. Regression models evaluation

Circumference and height collected at the end of the second growing season (Circum2 and Height2) were of overriding importance as compared to the number of sylleptic branches (Syll1) collected at the end of first growing season, pointed out by significant *p*-values of predictors (generally Circum2 and Height2 were significant) and by the parameters' weight ($b > c > d$) (Tab. 4.2). Only for BranchDM at CV 2004, Syll1 was a significant predictor. Coefficient of determination of the multiple regression analysis (R^2_{multi}) ranged from 0.66 to 0.98, with higher values for StemDM and TotalDM than BranchDM (0.69 and 0.66 at CV 2004 and MT 2004). Overall, the R^2_{multi} values were high for the coppiced sites (VT and SAV). No clear relationship was observed between the number of significant predictors and R^2_{multi} values. According to the values of average bias ($B_{\%}$), there was a distinct pattern in the overestimation of all biomass traits at all sites. Very low values of $B_{\%}$ were observed for StemDM and TotalDM (lower than 1%), with the exception of CV 2004 presenting the highest $B_{\%}$ observed for BranchDM (-7.00 %). Overall, for the BranchDM less accurate equations were observed. Values of standard error estimate ($SEE_{\%}$) showed the same behavior of $B_{\%}$, with greater spread for BranchDM (with a maximum value of 33.26%) than StemDM and TotalDM. Summarizing, it appeared to be the most accurate equation used to evaluate stem and total weight.

Table 4.2: Biomass equations for tree dry mass for the three biomass components in the five environments. Statistical significance of predictors x_1(Circum2), x_2 (Height2), and x_3 (Syll1) is given (*ns* = non significant, * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$). *a*, *b*, *c*, *d* values are parameters of general model $y=a+bx_1+cx_2+dx_3$. Statistical significance of the whole model (*P*-value.), coefficient of determination of the multiple regression analysis (R^2_{multi}), average bias ($B_{\%}$) and standard error estimate ($SEE_{\%}$) for each equation are also given.

			Importance of predictors							**Accuracy of estimation model**			
Env.	Trait	Transf.	x_1	x_2	x_3	*a*	*b*	*c*	*d*	*P* -value	R^2_{multi}	$B_{\%}$	$SEE_{\%}$
	StemDM	sqrt	***	***	*ns*	-14.835	1.589	0.052	-	***	0.79	-2.23	14.68
CV 2004	BranchDM	sqrt	***	*ns*	*	-6.689	2.139	-	0.127	***	0.69	-7.00	30.42
	TotalDM	sqrt	***	**	*ns*	-17.502	2.505	0.049	-	***	0.78	-3.01	17.06
	StemDM	sqrt	***	**	*ns*	-9.997	2.697	0.026	-	***	0.93	-0.68	9.88
MT 2004	BranchDM	ln	***	*	*ns*	5.017	0.251	-0.002	-	***	0.66	-1.96	26.39
	TotalDM	ln	***	*ns*	*ns*	5.338	0.191	-	-	***	0.81	-1.61	17.68
	StemDM	sqrt	***	***	-	-23.866	2.919	0.033	-	***	0.96	-0.37	6.82
SAV 2010	BranchDM	sqrt	***	***	-	14.175	2.930	-0.037	-	***	0.83	-2.79	20.55
	TotalDM	sqrt	***	*ns*	-	-8.187	4.122	-	-	***	0.95	-0.55	8.19
	StemDM	sqrt	***	***	-	-19.984	2.838	0.035	-	***	0.98	-0.15	8.65
VT 2010	BranchDM	sqrt	***	*	-	5.586	2.735	-0.025	-	***	0.91	-2.80	22.43
	TotalDM	sqrt	***	*	-	-13.990	3.859	0.016	-	***	0.98	-0.19	8.19
	StemDM	sqrt	***	***	-	1.49.2	0.324	0.005	-	***	0.98	-0.61	8.70
VT 2012	BranchDM	sqrt	***	**	-	4.198	2.900	-0.025	-	***	0.91	-4.99	33.26
	TotalDM	sqrt	***	*	-	1.433	1.339	0.004	-	***	0.97	-0.85	12.39

Analysis of covariance performed on the different multiple regressions (Tab. 4.3) showed two clear patterns; ANCOVA performed on different sites showed at least significant variance (*P*-value $\leq$ 0.05) between slopes (α) or between intercepts (z) (with an exception for BranchDM). Other than for ANCOVA performed in the same site (VT) no significant variance (*P*-value > 0.05) was observed for both α and z. Although the presence of no significant interaction (TotalDM, BranchDM), significant variance between intercepts was present and reveal a relevant variance between sites. For VT 2010 and SAV 2010, and for MT 2004 and CV 2004, it was not possible to use only one equation to estimate StemDM and TotalDM. Regression multiple models used in VT 2010 and VT 2012 did not show significant variance between α and z. Based on these results, it is possible to use the same equation to estimate stem, branches and total biomass in both years.

Table 4.3: Analysis of covariance among biomass estimation models. ANCOVA was performed between different sites with the same root/shoot ratio (R/S), and between different years for the same site. Significant continuous variables Circum2 (x_1) and Height2 (x_2), categorical variables (Env.) and their interaction (x_1×Env., x_2×Env) are shown. Significant statistic of variances between slopes (α) and between intercepts (z) of models are shown (*ns* = non significant, * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$). For the regression without significant variance of parameters, the unified equation is shown.

Env.	R/S	Traits	Significant effects				α	z	Equation
CV 2004 vs. MT 2004	R2-S2	StemDM	x_1	x_2	x_1×Env.	Env.	**		
		BranchDM	x_1			Env.	*ns*	***	
		TotalDM	x_1		x_1×Env.	Env.	**		
SAV 2010 vs. VT 2010	R3-S2	StemDM	x_1		x_2×Env.	Env.	*		
		BranchDM	x_1	x_2			*ns*	*ns*	$3.641+2.629x_1-0.019x_2$
		TotalDM	x_1			Env.	*ns*	***	
VT 2010 vs. VT 2012	R3-S2 R5-S2	StemDM	x_1	x_2			*ns*	*ns*	$-16.034+2.772x_1+0.031x_2$
		BranchDM	x_1	x_2			*ns*	*ns*	$7.109+2.922x_1-0.031x_2$
		TotalDM	x_1	x_2			*ns*	*ns*	$-8.748+3.947x_1-0.007x_2$

4.2.2. Comparison of POP5 parents and family mean values

To give an exhaustive description of relationships between genotypes variance and geographic position of sites, the following summary table and series of box-plots are exposed (Tab. 4.5., Fig. 4.2). Considering the total dry biomass (TotalDM), progeny showed the best performance in VT 2012, with the greatest variance between the extreme genotypes (8957 g of variance between the most and the least productive genotypes). VT 2012 showed greater medium tree weight than VT 2010, despite the different age of root system (respectively R5-S2 and R3-S2), and considering the different number of genotypes (129 and 158) and the mean number of observation per genotype (3.9 and 4.6). Conversely, the POP5 family showed the best yield performance in VT 2010 than VT 2012, with 14.9 t ha^{-1} $year^{-1}$ and 13.2 t ha^{-1} $year^{-1}$ of realized aboveground wood productivity (AWP_{real}) (Tab. 4.4). Comparing VT 2010 and SAV 2010 (R3-S2 for both sites), very similar genotypic means and variances were observed for the three biomass components. It was possible to observe two distinct groups that showed considerable variance in yield performance; a first group of sites with MT 2004 and CV 2004 (R2-S2, without coppicing) with a AWP_{real} of 6.1 t ha^{-1} $year^{-1}$ and 7.1 t ha^{-1} $year^{-1}$, and a second group of more productive sites (VT 2010, VT 2012 and SAV 2010)

characterized by coppicing to the end of the second growing season. The overall mean value of progeny productivity was 11.3 t ha^{-1} $year^{-1}$.

Table 4.4: Realized aboveground wood productivity (AWP_{real}) expressed in t ha^{-1} $year^{-1}$, for the five environments.

Env.	**F1**	**Poli**	**58-861**
CV 2004	7.1	1.9	5.8
MT 2004	6.1	4.2	5.0
SAV 2010	15.1	2.9	24.5
VT 2010	14.9	3.1	12.9
VT 2012	13.2	4.7	9.6
mean	11.3	3.4	11.6

Interesting results were observed for the percentage of branch weight respect to the total mass of clones (Fig. 4.2 B). In MT 2004 and CV 2004 were observed the greatest values of percentage (mean of 44.2% and 33%, respectively), moreover were observe large ranges of variance among genotypes (22.9% in MT 2004 and 21.6% in CV 2004). Otherwise, in VT 2010 and SAV 2010 were observed low mean values of proportion (27.7% and 29.2%, respectively), with a short range of variance (4.9% in VT 2010 and 7.9% in SAV 2010). In VT 2012 was observed a distinct behavior, with high values of percentage of branch biomass (35.8%) and genotypic means closed around overall mean.

For circumference and height (Fig.4.2 C and D), greater values were observed for VT and SAV environments than MT and CV. It was interesting to note that for VT 2012 and CV 2004 were observed closed values of Heigth2 mean (647.3 cm and 628.2 cm, respectively) and variance, but with considerable difference in Circum2 mean values (190.2 mm for VT 2012 and 144.5 mm for CV 2004). From the ANOVA, differences among genotypes resulted significant for all biomass traits, with the exception of Height1, Height2 and Circum2 collected in VT 2012.

Parent “Poli” showed, in all the environments, the lowest values of tree dimension and yield performance (overall mean value of 3.4 t ha^{-1} $year^{-1}$), with considerable transgressive segregation only in MT 2004. Circum2 and Height2 showed the same behavior observed for tree weight, with an exception at MT 2004, where the mean values of “Poli”, “58-861” and progeny were very close (Tab. 4.5). An overview of data showed same performances for circumference and height, despite the difference in sites fertility, year climatic conditions and roots age. The percentage of biomass

related to branches showed no clear trend, with different performances in the sites respect to family mean.

Parent “58-861” showed the greatest weight in SAV 2010 (8507 g) with low cases of transgressive segregation (Fig. 4.2 A). Very high value of AWP_{real} = 24.5 t ha^{-1} $year^{-1}$ were observed in the same site. In the other sites, weight and yield performances were closed around the family mean value,. Unlike to male parent, “58-861” showed more variance among the sites for all biomass traits, but like observed for progeny, it showed same mean values for Height2 in VT 2012 (632.6 cm) and CV 2004 (635.8 cm). In proportion to total weight, biomass of branches was higher in the southern sites (Fig. 4.2 B). Results of *t*-test performed between “Poli” and "58-861" showed significant variances for all the traits in the five environments, with the exception of biomass components in VT 2012 and for all traits sampled in MT 2004.

To explain the relationship between aboveground biomass allocation and site fertility, the percentage of branch weight respect to tree total biomass was plotted against the height (Height2) (Fig. 4.3). In SAV 2010 and VT 2010 it was possible observed a few direct proportionality between traits, which was null in VT 2012. Otherwise, in MT 2004 and CV 2004 was observed a widespread distribution of genotypes, so a lack of regression between traits. A total view of clones tested, showed a decrement of percentage of biomass of branch in response to an increment of height, and a semi-log regression explain with high accuracy (R^2=0.70 with *P*-value $\leq$ 0.001) this relationship.

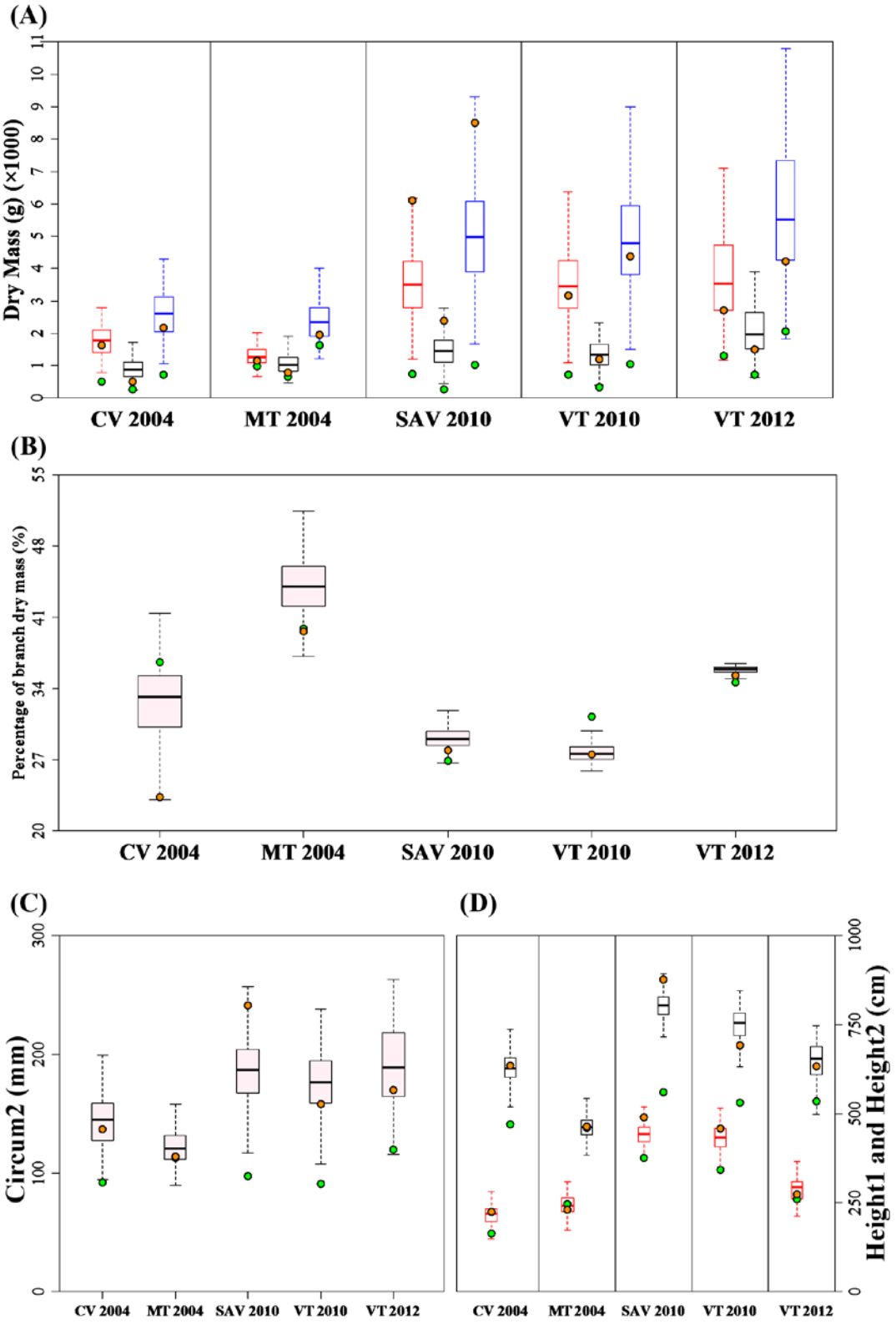

Figure 4.2: Genotypic variation of biomass traits. (A) Dry mass of stem (red boxes), branches (black boxes) and whole plant (blue boxes) in different environments. (B) Percentage of dry mass of branches respect to total weight of the plant. (C) Genotypic variation of circumference (Circum2) collected in the year of harvesting. (D) Height collected in the year of harvesting (Height2) (black boxes) and in the previous year (Height1) (red boxes). Mean values of northern parent "58-861" (orange circle) and southern parent "Poli" (green circle) is showed.

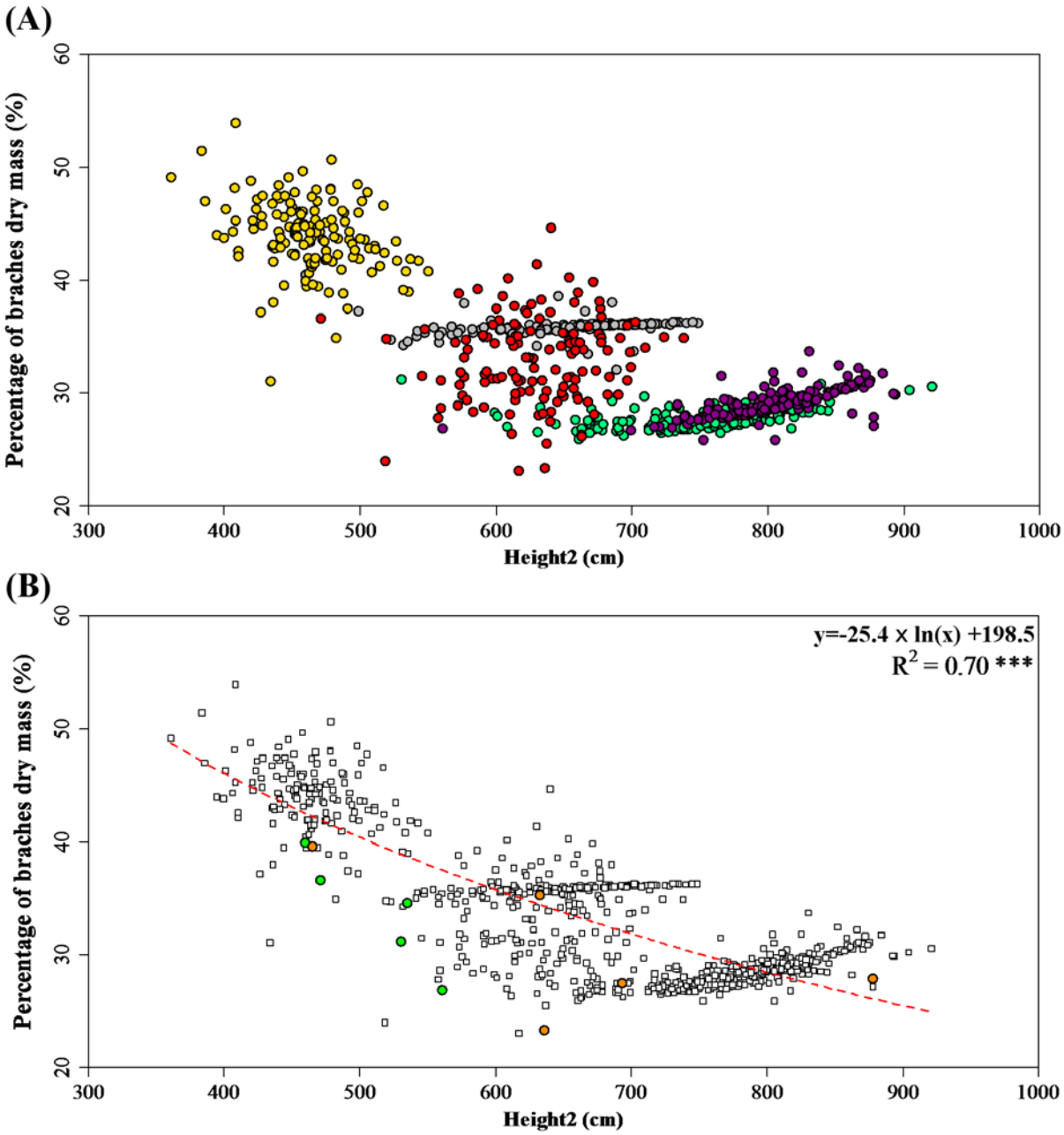

Figure 4.3: Relationship between height (Height2) and percentage of branches dry mass. (A) Genotypic mean of CV 2004 (red circles), MT 2004 (yellow circles), SAV 2010 (violet circles), VT 2010 (green circles) and VT 2012 (grey circles) and are shown separately. (B) Undifferentiated data are shown together (open white squares) "Poli" (green circles) and "58-861" (orange circles). A semi-log relationship between variables (y=-25.4×ln(x)+198.5) is shown (dashed red line) with coefficient R^2 and statistical significance of regression (*** = $P \leq 0.001$).

Table 4.5: Phenotypic performance of POP5 family of biomass traits: sampled height and circumference in year of biomass measures (Height2, Circum2) and height and number of sylleptic branches collected in the previous year (Height1, Syll1) are shown. Dry mass of stem (StemDM), branches (BranchDM) and of whole plant (TotalDM) are shown. Parent average values ("Poli", "58-861") ± standard errors (SE) and Student's *t*-test significance differences (*t*-test) between them are shown. Family genotypic variation with number of genotype analyzed (gen.), average number of individuals per genotype (obs.), population means (μ), and *F*-test significance differences (*F*-test) among genotypes for POP5 grown in the environments are shown. The significance level of the statistical tests is indicated as: *ns* = non significant, * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$.

			Family phenotypic variation						**Parents phenotypic variation**						
Envir.	**Trait**	**unit**	**μ**	**±**	**SE**	***F*-test**	**obs.**	**gen.**	**Poli**	**±**	**SE**	**58-861**	**±**	**SE**	***t*-test**
CV 2004	Height1	cm	214.9	±	1.5	***	4.9	154	163.6	±	13.5	224.4	±	21.5	*
	Syll1	-	31.8	±	0.4	***	4.9	132	41.6	±	5.5	17.2	±	4.1	**
	Height2	cm	628.2	±	2.4	***	4.8	150	470.8	±	17.4	635.8	±	33.0	**
	Circum2	mm	144.5	±	0.1	***	4.8	150	92.0	±	5.6	136.8	±	11.0	*
	StemDM	g	1780	±	25.7	***	4.8	150	498	±	53.2	1624	±	267.9	*
	BranchDM	g	895	±	18.5	***	4.9	130	265	±	44.8	505	±	144.5	*ns*
	TotalDM	g	2635	±	42.1	***	4.9	150	724	±	100.2	2164	±	408.2	*
MT 2004	Height1	cm	244.5	±	1.9	***	4.8	148	247.0	±	11.6	230.0	±	25.6	*ns*
	Syll1	-	49.9	±	0.6	***	4.7	122	82.5	±	3.1	26.2	±	7.1	*ns*
	Height2	cm	462.8	±	2.2	***	4.8	145	460.0	±	51.2	465.2	±	24.4	*ns*
	Circum2	mm	122.2	±	0.1	*	4.8	145	113.0	±	7.2	114.0	±	12.6	*ns*
	StemDM	g	1309	±	22.2	***	4.7	145	983	±	172.4	1155	±	218.9	*ns*
	BranchDM	g	1058	±	22.2	**	4.7	143	654	±	72.9	777	±	143.4	*ns*
	TotalDM	g	2387	±	45.3	***	4.7	143	1637	±	165.7	1958	±	321.9	*ns*
SAV 2010	Height1	cm	443.1	±	1.8	***	5.3	163	376.9	±	8.9	490.8	±	16.4	***
	Height2	cm	805.0	±	2.1	***	5.3	163	560.8	±	53.5	877.7	±	17.8	**
	Circum2	mm	189.2	±	0.2	***	5.3	163	97.2	±	12.6	241.4	±	14.0	***
	StemDM	g	3675	±	62.0	***	5.3	163	739	±	297.6	6112	±	734.6	***
	BranchDM	g	1545	±	30.1	***	5.3	163	276	±	114.5	2381	±	325.6	***
	TotalDM	g	5236	±	92.0	***	5.3	163	1025	±	411.6	8507	±	1027.7	***
VT 2010	Height1	cm	433.4	±	2.4	***	4.6	159	342.5	±	10.0	457.8	±	17.7	***
	Height2	cm	748.9	±	3.8	***	4.6	159	530.5	±	25.8	692.8	±	53.2	*
	Circum2	mm	177.2	±	0.2	***	4.6	158	91.1	±	7.6	158.4	±	18.8	**
	StemDM	g	3598	±	83.5	***	4.6	158	721	±	159.6	3163	±	605.4	**
	BranchDM	g	1402	±	36.1	***	4.6	158	324	±	55.7	1201	±	233.0	**
	TotalDM	g	5012	±	119.8	***	4.6	158	1041	±	213.8	4366	±	841.9	**
VT 2012	Height1	cm	287.1	±	2.6	*ns*	4.0	128	260.3	±	16	273.1	±	14.1	***
	Height2	cm	647.3	±	4.8	*ns*	3.9	121	535.1	±	26	632.6	±	26.1	*
	Circum2	mm	190.2	±	2.9	*ns*	3.8	124	120.1	±	13	170.1	±	13.7	*
	StemDM	g	3727	±	112.3	*	3.9	121	1303	±	306	2702	±	588.4	*ns*
	BranchDM	g	2087	±	63.2	*	3.9	129	712	±	176	1490	±	328.3	*ns*
	TotalDM	g	5806	±	172.1	*	3.9	129	2054	±	484	4215	±	894.6	*ns*

4.2.3. Within-site family variability and heritability

Low values of H_i^2 were generally observed in all environments for different traits (Tab. 4.6), whit the highest values observed for the number of sylleptic branches at the end of the first growing season (H_i^2 = 0.36) in MT 2004, and the lowest value for Height1 observed in VT 2012 (H_i^2 = 0.05). Generally, higher values of CV_G and CV_ε were observed for stem, branches and total dry mass than for circumference and height. This was considered an expected result given that biomass observations were extrapolated from linear regression of circumference and height. VT 2010 and SAV 2010 showed very similar CV_G values for each trait, and these results suggested like genetic effects ruled observed phenotypic variance with same extent in these two sites. Otherwise, higher CV_ε values were observed in VT 2010 than SAV 2010 and suggest like differences among clones of same genotype were larger at the first site, as a consequence of greater environmental influence. Respect to VT 2010, VT 2012 showed comparable values of CV_ε and pronounced decrement of CV_G for biomass components. As consequence of these CV values, heritability was very low. Concerning MT 2004 and CV 2004, in the letter site were observed values of H_i^2 closed between 0.25-0.27, conversely in MT 2004 more variance was observed between heritabilities, with higher value for Syll1 that suggests greater genetic control than for other traits.

Table 4.6: Genetic variance for biomass traits. Coefficients of Genetic (CV_G) and Residual (CV_e) Variation, individual (H^2_i) and genotypic (H^2_g) broad-sense heritability for the family grown in more environments are shown.

			Family genetic variation			
Env.	**Trait**	**unit**	**CV_G**	**CV_ε**	**H^2_i**	**H^2_g**
CV 2004	Height1	cm	8.46	16.87	0.20	0.56
	Syll1	-	18.12	29.12	0.27	0.65
	Height2	cm	5.06	9.07	0.26	0.63
	Circum2	mm	11.83	20.05	0.25	0.62
	StemDM	g	19.64	33.09	0.25	0.62
	BranchDM	g	26.67	43.77	0.27	0.65
	TotalDM	g	21.67	36.60	0.25	0.62
MT 2004	Height1	cm	7.21	19.65	0.13	0.43
	Syll1	-	16.98	22.64	0.36	0.72
	Height2	cm	5.33	10.96	0.19	0.53
	Circum2	mm	5.59	21.43	0.06	0.24
	StemDM	g	16.62	40.77	0.12	0.41
	BranchDM	g	16.70	51.08	0.08	0.29
	TotalDM	g	16.32	46.01	0.09	0.33
SAV 2010	Height1	cm	5.26	10.47	0.27	0.66
	Height2	cm	3.61	6.59	0.27	0.66
	Circum2	mm	12.79	20.61	0.27	0.66
	StemDM	g	27.12	41.19	0.28	0.68
	BranchDM	g	31.48	47.55	0.28	0.67
	TotalDM	g	28.34	42.89	0.28	0.67
VT 2010	Height1	cm	5.03	14.29	0.14	0.43
	Height2	cm	4.54	13.16	0.12	0.40
	Circum2	mm	11.58	29.19	0.13	0.42
	StemDM	g	25.90	57.86	0.13	0.41
	BranchDM	g	30.10	63.67	0.13	0.41
	TotalDM	g	27.03	59.42	0.13	0.41
VT 2012	Height1	cm	4.92	20.00	0.05	0.19
	Height2	cm	3.84	15.64	0.06	0.21
	Circum2	mm	8.22	32.15	0.06	0.20
	StemDM	g	18.01	62.94	0.06	0.21
	BranchDM	g	18.13	63.18	0.06	0.21
	TotalDM	g	17.71	61.87	0.06	0.21

4.2.4. Analysis of variance between environments and genotype stability

Genotype and environment effects were significant for the analyzed traits, expect the environment effect for StemDM between 2010 and 2012 in VT site (Tab. 4.7). Otherwise, interaction effect was significant for all the studied traits between MT 2004 and CV 2004, was often significant between VT 2010 and VT 2012 (except for Height1), and sometime between VT 2010 and SAV 2010 (for Height1 and BranchDM). Generally, values of the relative importance of G×E source were very low (the greatest value of 8.63% was observed for StemDM between MT 2004 and CV 2004).

G×E variance components observed in CV 2004 and MT 2004 sites, characterized by lack of coppicing at the end of the first growing season, were greater than values observed for the two other couples of environments. High values of σ_E^2/σ_P^2 (except for BranchDM and TotalDM) and significant values of ρ revealed that G×E interaction was determined by scale effects rather than changes in ranks among genotypes.

Between VT 2010 and SAV 2010, generally was observed a lack of significant interaction component (except for Height1 and BranchDM), with moderate values of rank coefficient (closed between 0.33 and 0.43) and very high significant p-value (i.e. to reject the H0 hypothesis, hence to accept an association of genotype rank values). Values of σ_E^2/σ_P^2 related to Height1, Circum2, StemDM, BranchDM and TotalDM were very low (maximum value of 2.83%, seen the similar mean performances of family in the sites. Furthermore, high values of σ_G^2/σ_P^2 for these couple of sites (SAV 2010 vs. VT 2010) suggested a greater genotypic variance than VT 2010 vs. VT 2012. For all traits, very high values of $\sigma_\varepsilon^2/\sigma_P^2$ were observed.

For Height1 studied in VT 2010 vs. VT 2012, high values of environment effect (72.16%) and low values of Spearman's coefficient (0.09) with not significant test value (i.e. to accept the H0 hypothesis, hence random changes of genotype rank occurred) suggested both ranking and scale effects among years. Moreover, low value of σ_G^2 relative importance (1.10%) suggested that for both the rotation (2009-2010 and 2011-2012), Height1 genotype variance were reduced. Results observed for height collected to the end of second growing season (Height2) of each rotation, showed a drastic reduction of σ_E^2/σ_P^2 (35.77%) and a sensible rising of σ_G^2/σ_P^2 and $\sigma_\varepsilon^2/\sigma_P^2$ (respectively 5.81% and 55.37%). For biomass components were observed very high values of $\sigma_\varepsilon^2/\sigma_P^2$ (at least 77%) due to very large variance observed among clones performance, thus environment effects were hidden and has not been interpreted. Despite this, substantial values of σ_G^2/σ_P^2 (at least 8.97%) were observed for stem, branches and total biomass.

In terms of relative environment ecovalence, VT 2010 showed a lower contribution in both cases where was considered than other environments (VT 2012 and SAV 2010). Considering VT 2010 and VT 2012, relative contribution was very unbalanced to second years (W^2_2 = 63.5 %). Different distributions of ecovalence were observed for the traits studied between MT 2004 and CV 2004, with equal partition between sites (50% for CV 2004 - 50% for MT 2004. A low percentage of genotype (closed values between 11.8% and 15.6%) accounting 50% of the total G×E observed ($W^2_{50\%}$). Furthermore, the greatest part of genotypes (closed values between 58.6% and 70.1%) were below the mean values of W^2 (lower than 1% for all traits).

Table 4.7: Relative importance of genetic (σ^2_G), environment (σ^2_E), genotype by environment ($\sigma^2_{G\times E}$), and residual (σ^2_ε) effects in the phenotypic variation (σ^2_P), with their statistic significance. Spearman's rank correlation coefficient (ρ) with his statistic significance (*P*-value) and relative ecovalence between the first (W^2_1) and the second (W^2_2) environment are shown. Percentage of genotypes accounting 50% of the total G × E observed ($W^2_{50\%}$) and were below ecovalence mean value ($<\mu_w$) are shown. Note: *ns* = non significant, * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$, - = too low to be quantified.

			Variance components (%)								Spearman		Relative Ecovalance (%)			
Env.	Trait	unit	σ^2_E/σ^2_P		σ^2_G/σ^2_P		$\sigma^2_{G\times E}/\sigma^2_P$		$\sigma^2_\varepsilon/\sigma^2_P$		ρ	*P*-value	W^2_1	W^2_2	$W^2_{50\%}$	$<\mu_w$
CV 2004 vs. MT 2004	Height1	cm	17.64	***	8.23	***	4.07	*	70.04		0.30	***	50.2	49.8	11.8	70.1
	Syll1	-	51.68	***	10.32	***	4.56	***	33.42		0.32	***	52.7	47.4	15.3	66.9
	Height2	cm	80.61	***	2.18	***	2.19	***	15.00		0.28	***	51.6	48.5	12.4	67.9
	Circum2	mm	22.03	***	7.27	***	7.68	***	63.01		0.44	***	51.5	48.5	14.5	65.0
	StemDM	g	22.02	***	7.86	***	8.63	***	61.46		0.28	***	52.5	47.5	12.4	67.2
	BranchDM	g	3.98	***	9.99	***	7.53	**	78.40		0.25	**	51.2	48.8	14.2	66.7
	TotalDM	g	2.55	***	10.27	***	7.24	**	79.90		0.29	***	50.9	49.1	12.7	64.8
SAV 2010 vs. VT 2010	Height1	cm	0.67	**	15.30	***	3.25	*	80.76		0.33	***	43.8	56.2	13.3	69.8
	Height2	cm	15.18	***	13.81	***	2.52	*ns*	68.46		0.37	***				
	Circum2	mm	2.83	***	16.21	***	2.86	*ns*	78.08		0.42	***				
	StemDM	g	0.45	*	16.56	***	3.05	*ns*	79.93		0.43	***				
	BranchDM	g	2.53	***	15.46	***	3.77	*	78.22		0.43	***	44.3	55.7	12.0	68.9
	TotalDM	g	1.07	***	16.06	***	3.18	*ns*	79.67		0.43	***				
VT 2010 vs. VT 2012	Height1	cm	72.16	***	1.10	***	1.68	*	25.04		0.09	ns	36.5	63.5	15.6	58.6
	Height2	cm	35.77	***	5.81	***	3.02	*ns*	55.37		0.23	*				
	Circum2	mm	1.88	***	10.71	***	1.80	*ns*	85.59		0.31	***				
	StemDM	g	-	*ns*	10.27	***	2.20	*ns*	87.52		0.37	***				
	BranchDM	g	12.03	***	8.97	***	1.74	*ns*	77.25		0.38	***				
	TotalDM	g	1.52	***	10.03	***	2.24	*ns*	86.19		0.38	***				

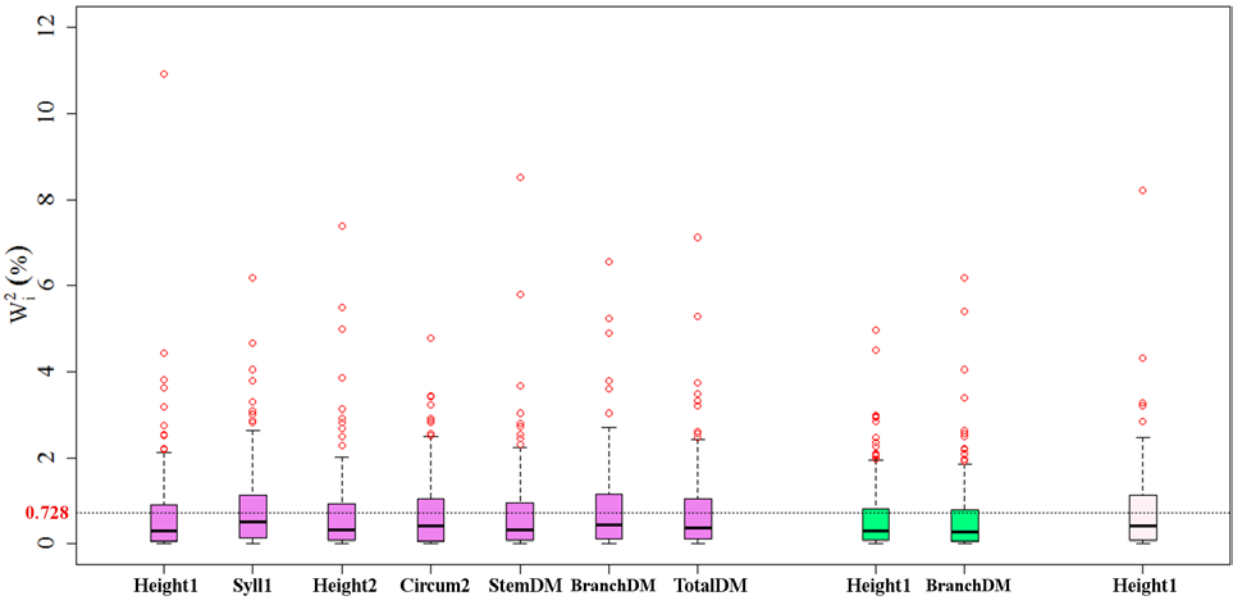

Figure 4.4: Genotype distribution of the relative genotype ecovalence (W^2_i) for the different biomass and biometric traits with significant G×E effect. Results for CV 2004 vs. MT 2004 (violet), SAV 2010 vs. VT 2010 (green) and VT 2010 vs. VT 2012 (lavender) are shown. Dotted horizontal line represent overall mean ecovalence value among environments.

4.3. Bud flush phenology

4.3.1. Temperature during the rest season and bud flush period

Cumulative Minimum Temperature below 5°C (CMT_5) and Cumulative Average Temperature above 10°C (CAT_{10}) are useful parameters to describe the trend of the temperature during the period and their influence on the bud flush process. CMT_5 showed clear different cold accumulations among northern (CV and SAV) and southern locations (MT and VT) during the rest season (Fig. 4.5 A). Northern sites reached values of 890°C and 1146°C versus lower values of 500°C recorded in the central Italy. Furthermore, in northern sites, CMT_5 was greater than CAT_0, and this suggests like climate was more continental in this areas then central zones of Italy. Otherwise, high values of CAT_0 in MT and VT, and positive accumulation values of CAT_{10} since November, 1st suggested a temperate climate that allows mild temperatures during the winter. Regarding heat and cold accumulations from March, 1st to April 30 (Fig. 4.5 B), CV and SAV showed greater increments for CMT_5 (158°C and 180°C) than CAT_{10} (69.3°C and 88.5°C), while for MT and VT 2012 was observed an inverse situation with increments of CAT_{10} greater than increments of CMT_5 (122.7°C and 124.1°C versus 54.2°C and 39.7°C). For VT 2010, characterized by a significant drop in temperature occurred during bud flush period, increments of CAT_{10} and CMT_5 were very similar (93.5°C and 96.7°C).

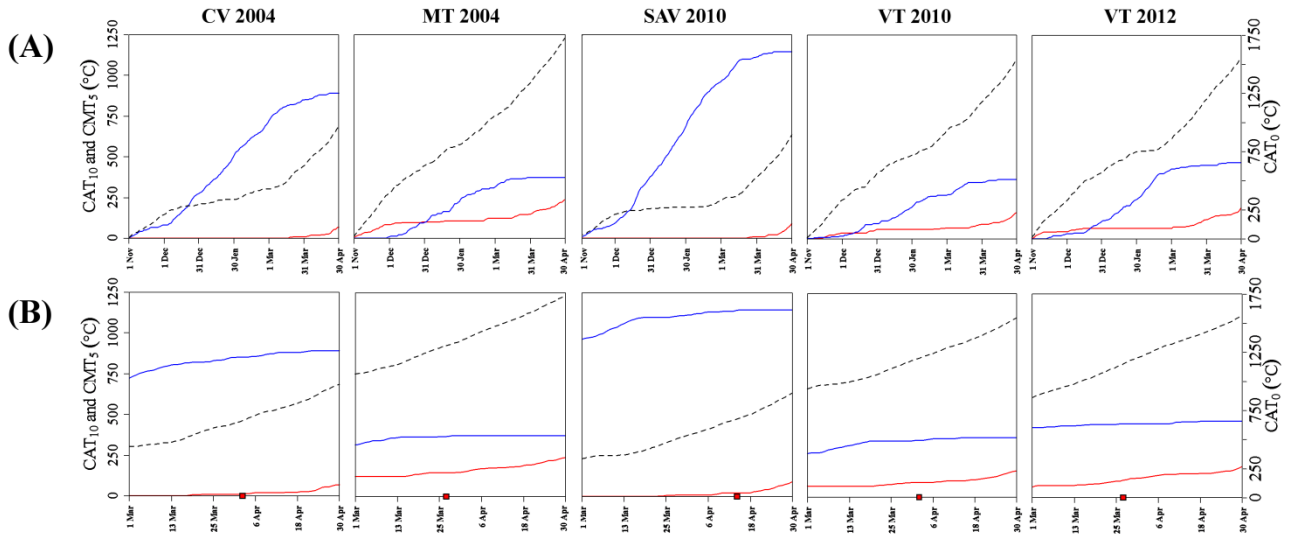

Figure 4.5: Accumulations of climatic parameters in the five environments. (A) CAT_{10} (red line), CMT_5 (blue line) and CAT_0 (black dashed line) calculated from November, 1st to April 30. (B) The same parameters are shown from March 1st to April 30. CAT_{10} and CMT_5 are plotted against the left vertical axis, while CAT_0 is plotted against right vertical axis. Overall mean family of bf_date1.5 (red square) is shown.

About trends of temperature observed during buds and leaves phenology (Fig. 4.6), VT 2010 showed a slow continuous temperature reduction from the beginning to the end of process, with daily mean temperature lower 10°C for 6 days of the total bud flush duration. In April, 5th (DOY 95) and April, 11th (DOY 101) were recorded the lowest mean daily temperatures during the process (8.5°C and 8.21°C, respectively), in correspondence to central stages of bud flush (bf_date_1.5 and bf_date_2). Conversely, daily mean temperature in VT 2012 was always above 10°C for whole phenology period, with a rising trend through the first part of the process (from bf_date_0.5 to bf_date_1.5). In SAV 2010 were recorded large fluctuations for mean and maximum daily temperatures (from 6.9°C to 16.1°C and from 8.8°C to 26°C), as expected in a continental climate location with large diurnal and seasonal ranges of temperature (Duckson 1987). In VT site, minimum daily temperature trend recorded in 2010 showed lower variance than 2012, in particular in the first part of the process (from bf_date05 to bf_date1.5). In VT 2012, range of variation of minimum temperatures was greater than VT 2010, with quick changes of temperature closed between 3°C and 10°C.

Different duration of the total bud flush process was observed in the three environments (Fig. 4.5). VT 2010 showed longer total duration of process (for both progeny and parents with overall mean of 21.8 days), than VT 2012 and SAV 2010 (overall mean of 17.4 and 19.1 days, respectively). Parent “Poli”, for both VT trials, required more time than progeny and “58-861”, to complete leaves elongation. The largest gap of 6 days was observed in VT 2010 between “Poli” (25.3 days) and “58-861” (19.3 days). In all sites, parent “58-861” showed a delay in the buds swelling (bf_date_0.5) respect to “Poli” (6-7 days) and respect to F_1 progeny (4-5 days).

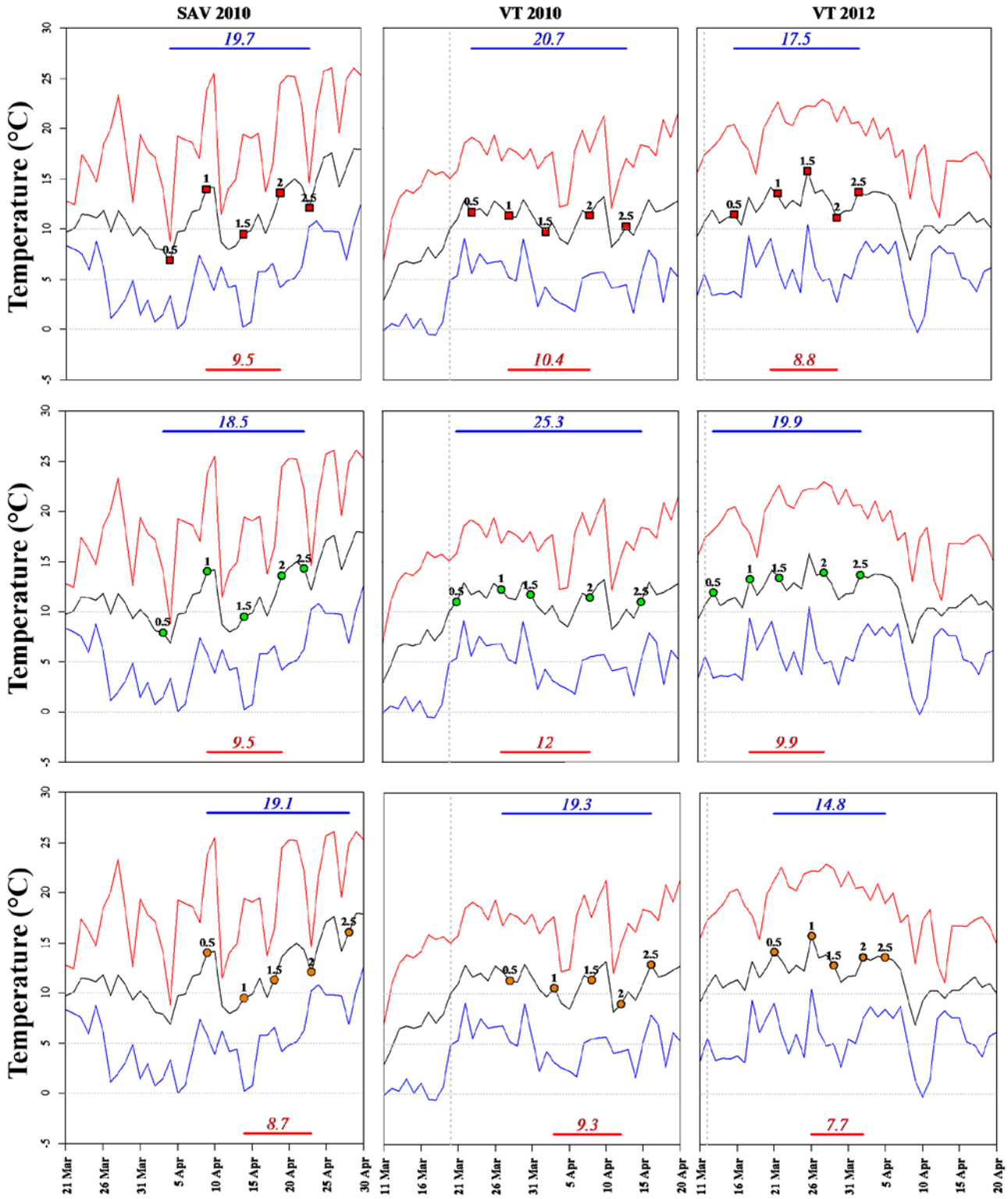

Figure 4.6: Temperatures trends during the bud flush. Mean values of bud flush stages of offspring (red squares), male parent “Poli” (green circles) and female parent “58-861” (orange circles) are plotted together daily mean temperatures (black line), daily maximum temperatures (red line) and daily minimum temperatures (blue line). Subprocess1 and total bud flush durations are shown respectively on the bottom (red bar and italic label) and on the top (blue bar and italic label) of each graph. For VT 2010 and VT 2012, the first day of growing season (GS) is plotted (vertical dotted gray line).

Beginning of growing season (GS) was determined like a period of at least 15 days with mean temperature equal or higher than a selected threshold (Perini et al. 2004). Considering a reference temperature of 10°C, GS was identified in March, 20th (DOY 79) for VT 2010 and in March, 12th (DOY 72) for VT 2012 (Fig. 4.5). Regrettably in SAV 2010, due to large fluctuations of mean temperatures, it was not possible to propose a threshold temperature and to found a GS value, according to Perini's definition.

In both the years 2010 and 2012, "Poli" and F_1 progeny showed a similar gap of days between GS beginning and the first stage of bud flush (bf_date0.5) (Tab. 4.8). Moreover for these genotypes, very similar values of heat accumulation (CAT_{10} calculated on these time intervals) corresponded, with same values (5.52°C and 5.69°C) for F_1 progeny. Only 0.97°C and 2.59°C were accumulated for "Poli", and this suggested like male parent was high vulnerable to a specify heat sum threshold. Parent "58-861" showed the same behavior between years, that is a greater delay in days and heat requirements than other genotypes.

Table 4.8: Time interval (gap) between the first day of growing season (GS) and the mean day of bud flush beginning (bf_date0.5) are showed. Cumulative average temperature above 10°C (CAT_{10}) was estimated for these time interval.

	VT 2010				**VT 2012**			
	GS (DOY)	**bf_date0.5 (DOY)**	**gap (DOY)**	**CAT_{10} (°C)**	**GS (DOY)**	**bf_date0.5 (DOY)**	**gap (DOY)**	**CAT_{10} (°C)**
Poli		80	1	0.97		73	1	2.59
58-861	79	86	7	13.86	72	81	9	17.67
F1		82	3	5.52		76	4	5.69

4.3.2. Phenotypic dynamics and variation in bud blush traits

In order to describe the observed trend of bud opening and leaf development of the whole family, genotypic mean of each genotype were plotted (Fig. 4.7). Horizontal dashed lines show the first (bf_date1) and the last (bf_date2) stages of leaf initiation duration (subprocess1). Moreover the bf_date1.5 variance, variance of bf_date0.5 was shown. Bud swelling was considered a stage of special interest, because was the first visible manifestation of bud flush phenology, usable as signal for perception of critical heat accumulation. Unfortunately for a VT 2010, temperatures triggered the process ahead of its awaited time, so it was not possible to collect related data to dormant buds (date0) for all the genotypes. Using the smoothing procedure, it was possible to recover these

missed data. Only 6 genotypes of progenies were available for SAV 2010, so their performance was not considered representative of genotypic variance of the whole POP5 family. Data collected in SAV 2010 was useful to compare the parent performances among contrasting environments.

Generally, parent “58-861” showed a considerable delay for all analyzed phases, except in VT 2010 where “Poli” and a low number of offspring genotypes (13) delayed between bf_date2 and bf_date2.5, and ended the process after the northern parent. However, parent “58-861” showed a physiological delay to detect the thermal threshold (bf_date0.5) and to break bud’s scales (bf_date1).

Differently, the southern parent “Poli” showed different behaviors in the environments. In VT 2012, male parent was earlier than most F_1 genotypes to reach phases from bf_date0.5 to bf_date2. Not negligible transgressive segregation of some genotypes (14) during the leaves unfolding (from bf_date2 to bf_date2.5) occurred for “Poli”. Less stable was the dynamics of “Poli” in VT 2010, with a considerable number of genotypes which showed transgressive segregation for all the stages, and 153 genotypes which ended bud blush before male parent.

An overall vision of bud flush stages showed greater variance in VT 2010 than VT 2012 (Tab. 4.8). In VT 2010 a gap of 13 days between the earliest and the latest genotype (genotype 67 at DOY 85 and parent “58-861” at DOY 98, respectively) was observed for bf_date1.5. For the same trait, a difference of 9 days was observed in VT 2012 (genotype 137 at DOY 81 and genotype 165 at DOY 90). About bf_date0.5, was observed greater variance in VT 2012 than VT 2010, with a gap of 10 days between the earliest genotype (137, DOY 71) and the latest genotype (“58-861”, DOY 81). In VT 2010 was observed a genotypic mean variability of 9 days (genotype 67 at DOY 78 and parent “58-861” at DOY 86). In VT 2012, if genotype 137 was considered like an outlier, range of variation between the earliest and the latest genotype would be 9 days for bf_date0.5, bf_date1 and bf_date1.5. Concerning thermal parameters, it was observed greater values of CMT_5 in VT 2010 than VT 2012, which corresponded with greater values of DOY durations at 2010 than 2012. A less clear relationship between CAT_{10} accumulations and DOY durations was observed for both the years.

Table 4.8: Date in which the earliest and latest genotypes reached each phase expressed in DOY, CAT_{10} and CMT_5. The intervals were calculated as the difference between the two extreme clones.

		The earliest genotype			**The latest genotype**			**Gap between genotypes**		
Envir.	**Traits**	**DOY**	**CAT_{10}**	**CMT_5**	**DOY**	**CAT_{10}**	**CMT_5**	**DOY**	**CAT_{10}**	**CMT_5**
VT 2010	bf_date0.5	78	69.7	344.5	86	83.6	344.6	8	13.9	0.1
	bf_date1	81	73.6	344.6	93	91.7	350.0	12	18.2	5.5
	bf_date1.5	85	81.4	344.6	98	95.1	358.3	13	13.7	13.8
	bf_date2	90	90.7	344.7	104	101.1	363.9	14	10.4	19.2
	bf_date2.5	99	97.6	358.3	111	117.1	366.2	12	19.5	7.9
VT 2012	bf_date0.5	71	74	439	81	91.6	448.3	10	17.6	9.3
	bf_date1	77	80.1	448.3	86	108	450.6	9	27.9	2.3
	bf_date1.5	81	91.6	448.3	90	119.4	453.1	9	27.8	4.8
	bf_date2	86	108	450.6	94	130	453.1	8	22	2.5
	bf_date2.5	93	126.6	453.1	97	140.8	453.1	4	14.2	0

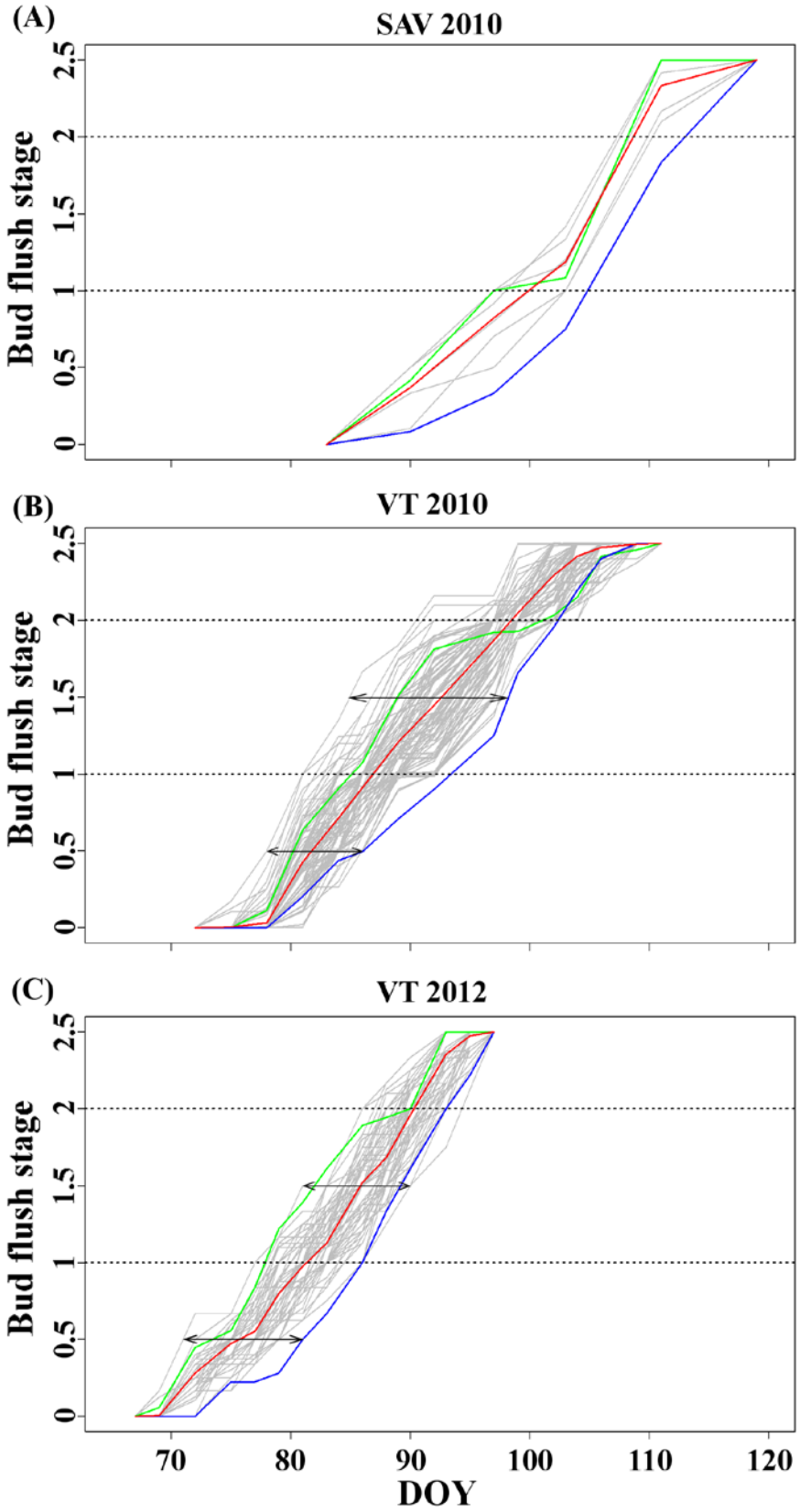

Figure 4.7: Observed trend of bud opening and leaf formation at the three environments (A) SAV 2010, (B) VT 2010 and (C) VT 2012. Genotypic means are showed for each F_1 genotype (grey lines), parent "58-861" (blue line) and parent "Poli" (green line). The POP5 family mean is indicated with a red line. Genotypes variance of perception of threshold heat accumulation (bf_date0.5) and leaf initiation (bf_date1.5) are showed (double arrow).

An overlapping between observed bud flush trends with daily increase of thermal parameters, showed interesting results concerning relationship between daily changes dynamics and temperatures during the process (Fig. 4.8). For VT 2010 from DOY 80 to DOY 90, without CMT_5 increment, were observed an untroubled progression (i.e. linear trend without relevant slope's changes) through stages, for "Poli", "58-861" and the progeny. After DOY 90, at the beginning of CMT_5, parent "Poli" responded with a drastic lag in development, than it was observed until the end of leaf formation. Instead, dynamics of parent "58-861" and F_1 progeny, not were affected by CMT_5 rising.

In VT 2012, for bf_date0.5 were observed the greatest phenotypic variance. Both a relevant heat accumulation (CAT_{10} of 13.52°C) and cold accumulation (CMT_5 of 9.29°C) were observed during buds swelling (from DOY 71 to DOY 81), which corresponded a lag to reach bf_date0.5, in particular for parents "Poli" and "58-861". In the further stages were observed comparable results with VT 2010, with greater lag for "Poli" than other genotypes observed between bf_date1.5 and bf_date2. In SAV 2010, CMT_5 increment was observed during the first phases (from DOY 83 to DOY 105 with CMT_5 of 45.7°C) corresponded to the slowest section of development. Differently in the second part of the process (from DOY 106 to DOY 119), in response to a high heat accumulation (CAT_{10} of 68°C), was observed a process speed similar to VT 2010 and VT 2012.

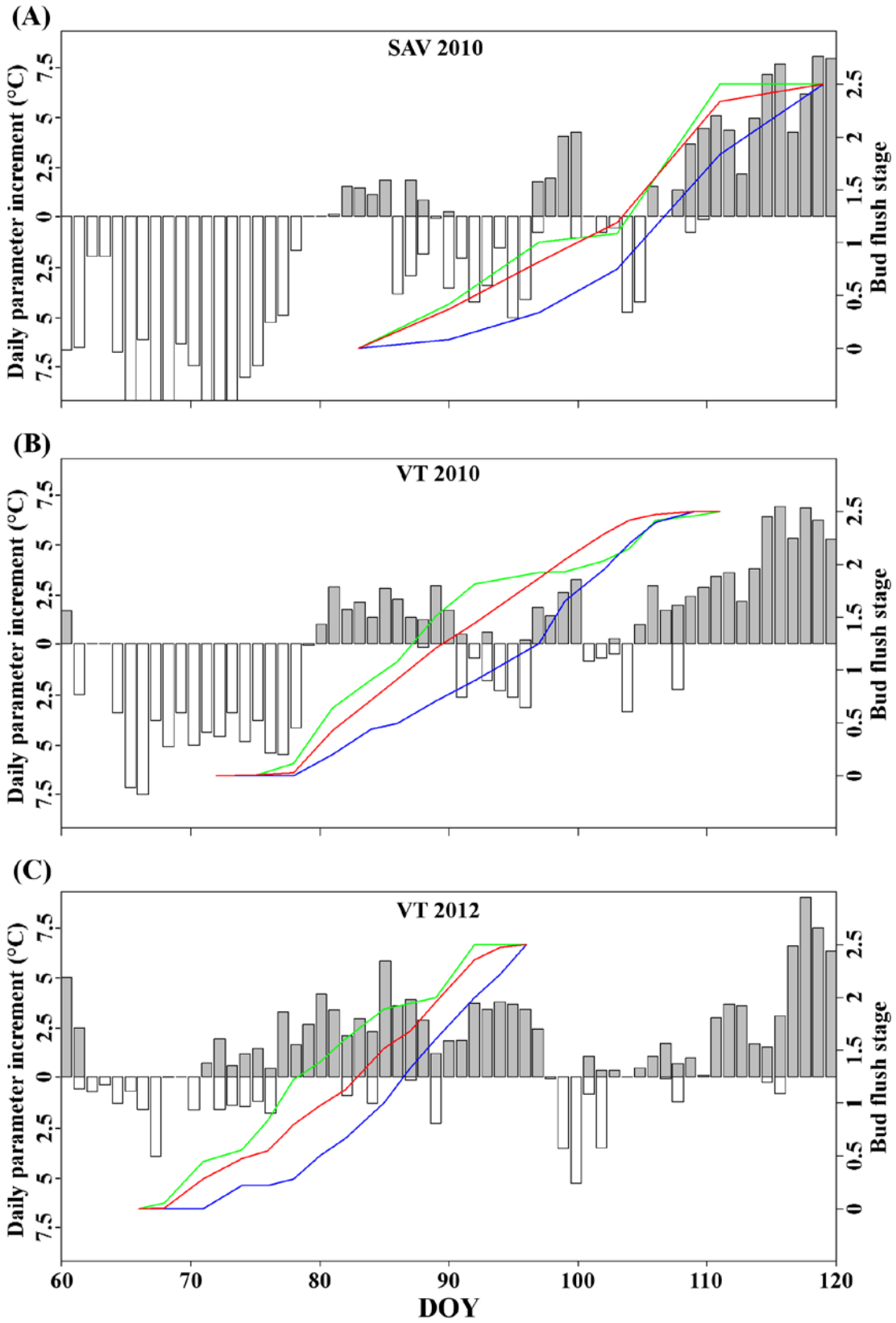

Figure 4.8: Observed trends of bud flush and CAT_{10} and CMT_5 daily increase. CAT_{10} daily increments are draw in the upper side of graph (grey bars), while CMT_5 daily increments are draw in the lower side (white bars). Genotypic means of process dynamics are showed for parent "58-861" (blue line), parent "Poli" (green line) and the whole F_1 progeny (red line). (A) SAV 2010, (B) VT 2010, (C) and VT 2012 are shown.

4.3.3. Comparison of POP5 parents and family mean values

In order to give an exhaustive description of relationships between genotypes variance and temperature effects on durations, the following summary table and series of box-plots are exposed. Due to the limited number of genotypes available in SAV 2010, only genotypic variances of VT 2010 and VT 2012 were plotted.

Data showed different total duration of bud flush for F_1 progeny, amounting to 20.7 and 17.4 days for VT 2010 and VT 2012 environments, respectively. Concerning the temperature accumulations, values of CAT_{10} equal to 24.4°C and 47°C and CMT_5 of 19.4°C and 6.8°C were observed in VT 2010 and VT 2012, respectively. Concerning the six F_1 genotypes scored in SAV 2010, total bud flush duration was 19.7 days, corresponding to 41.8°C of CAT_{10} and to 16.4°C of CMT_5. In VT 2010 and VT 2012, it was possible to observe similar ranges of genotypic variances for onset-of-stage date, with a normal distribution of genotypes around family mean (Fig. 4.9 A). A mean variance of 7 days, to reach each bud flush stage, was observed between progeny in VT 2012 (the earliest) and progeny in VT 2010 (the latest). Only 4°C of difference were observed between CAT_{10} threshold reached for bf_date0.5 in VT 2012 (81.1°C) and VT 2010 (77.1°C) (Fig. 4.9 B). Between VT 2012 and MT 2004, concerning bf_date1.5 expressed in DOY (Tab. 4.9), the same progeny mean (86.0 DOY) and closed values for "Poli" (82.2 DOY in VT 2012 and 83.8 DOY in MT 2004) and "58-861" (89.4 DOY in VT 2012 and 89.6 DOY in MT 2004) were observed. Despite these results, in MT 2004 for bf_date1.5 were accumulated more CAT_{10} than VT 2012 (approximately 40°C of variance) and less CMT_5 (approximately 80°C of variance).

Duration of bf_date0.5 and bf_date1 expressed in DOY showed variance and mean values highly comparable between years. Afterwards, in VT 2010 the duration of bf_date1.5 and bf_date2 showed mean and variances values greater than results found in VT 2012. It was important to highlight that family variances of stage durations were not significant in VT 2012 for DOY (all durations), for CAT_{10} (accumulation during bf_date1 and bf_date1.5) and for CMT_5 (accumulation during bf_date0.5 and bf_date1). Relating to heat (CAT_{10}) and cold accumulations (CMT_5) occurred in each stage, greater variance was observed for CAT_{10} climate parameter in VT 2012 than in VT 2010. Opposite situation happened for CMT_5 with greater values in VT 2010 than in VT 2012 (Fig. 4.9 C), this second year was characterized by a very low value of cold accumulation (only 6.8°C cumulated in 17.4 days). About the variance among progeny (*F*-test), significant levels were found for most of the traits.

Concerning “Poli” the total duration of the bud flush process in VT, it was slower than its offspring with 25.4 days in 2010 and 19.8 days in 2012, corresponding to 30.5°C and 49°C of CAT_{10}, respectively. Despite the 6.8 days of difference between VT 2010 and VT 2012 for the onset of bud phenology (bf_date0.5), closed values of CAT_{10} were observed with 74.3°C in VT 2010 and 77.6°C in VT 2012. In VT 2010, the greatest values of durations were recorded for bf_date1.5 (7.9 days) and for bf_date2 (7.7 days) corresponding to the greatest cold accumulation values (9.1°C and 4.9°C, respectively) observed in the year. Variances among stage durations expressed in DOY were lower in VT 2012 than in VT 2010, and among “Poli”, “58-861” and F_1 progeny, the lower variance for durations were observed for VT 2012 than VT 2010. About CMT_5, very similar values were observed among the two parents and the progeny for VT 2010, VT 2012 and SAV 2010. Concerning the bf_date1.5 in MT 2004 and CV 2004, not significant differences were found with respect to female parent.

Parent “58-861” showed a total duration of the bud flush process more similar to progeny than the male parent "Poli", with duration of 19.2 and 19 days and a related heat accumulation of 20.6°C and 63.9°C in VT 2010 and in SAV 2010, respectively. Almost the twice of CMT_5 total accumulation was observed in SAV 2010 for “58-861” (63.9°C) by comparison with “Poli” (33.7°C). In VT 2012, it showed lower duration (14.8 days) than F_1 progeny and “Poli”, accounting a heat sum of 44.3°C. About the process onset (bf_date0.5), a difference of 5.7 DOY between years (VT 2010 and VT 2012), equivalent to a heat accumulation of 7.7°C, were observed. Concerning bf_date1.5, in SAV 2010 and CV 2004 similar values of DOY (108.6 versus 106.2), and great variance for CAT_{10} (36.2°C versus 21.8°C) and CMT_5 (1140.1°C versus 880.6°C) were observed.

Table 4.9: Phenotypic performance of POP5 family for the onset of stage and duration. Parents mean ("Poli", "58-861") ± standard errors (SE) and Student's *t*-test significance differences (*t*-test) between them are given. Family genotypic variation with number of genotypes analyzed (gen.), average number of individuals per genotype (obs.), population means (μ), and *F*-test significance difference (*F*-test) among genotypes for POP5 grown in the environments are shown. The significance level of the statistical tests is indicated as: *ns* = non significant, * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$.

	CV 2004												
	Family phenotypic variation						**Parent values**						
Trait	**μ**	**±**	**SE**	***F*-test**	**obs.**	**gen.**	**Poli**	**±**	**SE**	**58-861**	**±**	**SE**	***t*-test**
bf_date1.5_DOY	92.2	±	0.13	***	4.93	154	89.6	±	2.76	106.2	±	0.48	**
bf_date1.5_CAT_{10}	13.1	±	0.13	***	4.93	154	11.7	±	1.73	21.8	±	0	**
bf_date1.5_CMT_5	850.22	±	0.30	***	4.93	154	845.16	±	5.79	880.6	±	0	**
	MT 2004												
	Family phenotypic variation						**Parent values**						
Trait	**μ**	**±**	**SE**	***F*-test**	**obs.**	**gen.**	**Poli**	**±**	**SE**	**58-861**	**±**	**SE**	***t*-test**
bf_date1.5_DOY	86.0	±	0.16	***	4.83	148	83.8	±	2.52	89.6	±	3.17	*ns*
bf_date1.5_CAT_{10}	148.51	±	0.23	***	4.83	148	144.79	±	2.83	153.8	±	6.13	*ns*
bf_date1.5_CMT_5	369.79	±	0.13	***	4.83	148	368.67	±	1.88	372.7	±	1.56	*ns*

Table 4.9 (continued): Phenotypic performance of POP5 family for the onset of stage and duration. Parents mean ("Poli", "58-861") ± standard errors (SE) and Student's *t*-test significance differences (*t*-test) between them are given. Family genotypic variation with number of genotypes analyzed (gen.), average number of individuals per genotype (obs.), population means (μ), and *F*-test significance difference (*F*-test) among genotypes for POP5 grown in the environments are shown. The significance level of the statistical tests is indicated as: *ns* = non significant, * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$.

	SAV 2010											
	Family phenotypic variation						**Parent values**					
Trait	**μ**	**±**	**SE**	***F*-test**	**obs.**	**gen.**	**Poli**	**±**	**SE**	**58-861**	**±**	**SE**	***t*-test**
bf_date0.5_DOY	93.7	±	0.31	***			93.4	±	0.35	99.3	±	0.77	***
bf_date1_DOY	99.4	±	0.33	***			99.4	±	0.44	104.5	±	0.58	***
bf_date1.5_DOY	104.2	±	0.34	***	5.5	6	104.3	±	0.42	108.6	±	0.52	***
bf_date2_DOY	108.9	±	0.34	***			108.8	±	0.40	113.1	±	0.59	***
bf_date2.5_DOY	113.4	±	0.60	***			111.9	±	0.89	118.3	±	0.67	***
duration0.5_DOY	5.7	±	0.08	*ns*			6.0	±	0.15	5.2	±	0.28	*
duration1_DOY	5.1	±	0.07	*ns*	5.5	6	4.9	±	0.08	4.1	±	0.17	***
duration1.5_DOY	4.7	±	0.11	*ns*			4.5	±	0.07	4.6	±	0.14	*ns*
duration2_DOY	4.5	±	0.31	*			3.1	±	0.58	5.2	±	0.51	*
subproc1_DOY	9.5	±	0.17	*ns*	5.5	6	9.4	±	0.08	8.7	±	0.27	*
total duration_DOY	19.7						18.5			19.1			
bf_date0.5_CAT_{10}	9.3	±	0.24	***			8.9	±	0.27	14.4	±	0.84	***
bf_date1_CAT_{10}	16.4	±	0.40	***			15.9	±	0.48	24.8	±	1.38	***
bf_date1.5_CAT_{10}	24.5	±	0.60	***	5.5	6	23.8	±	0.75	36.2	±	1.92	***
bf_date2_CAT_{10}	34.3	±	0.91	***			33.4	±	1.18	50.4	±	2.46	***
bf_date2.5_CAT_{10}	51.1	±	3.35	***			42.6	±	4.92	78.3	±	3.69	***
duration0.5_CAT_{10}	7.1	±	0.17	***			7.0	±	0.23	10.4	±	0.56	***
duration1_CAT_{10}	8.1	±	0.20	***	5.5	6	7.9	±	0.28	11.4	±	0.57	***
duration1.5_CAT_{10}	9.9	±	0.31	***			9.6	±	0.43	14.2	±	0.63	***
duration2_CAT_{10}	16.8	±	2.51	***			9.2	±	3.84	27.9	±	3.04	**
subproc1_CAT_{10}	18.0	±	0.51	***	5.5	6	17.5	±	0.71	25.6	±	1.16	***
total duration_CAT_{10}	41.9						33.7			63.9			
bf_date0.5_CMT_5	1130.0	±	0.54	**			1129.2	±	0.67	1134.7	±	0.27	***
bf_date1_CMT_5	1134.8	±	0.16	***			1134.8	±	0.20	1137.4	±	0.31	***
bf_date1.5_CMT_5	1137.8	±	0.20	***	5.5	6	1138.3	±	0.26	1140.1	±	0.30	***
bf_date2_CMT_5	1141.2	±	0.20	***			1141.7	±	0.25	1143.3	±	0.25	***
bf_date2.5_CMT_5	1146.4	±	0.00	-			1146.4	±	0.00	1146.4	±	0.00	-
duration0.5_CMT_5	4.3	±	0.51	*			5.6	±	0.59	2.7	±	0.15	***
duration1_CMT_5	3.0	±	0.10	*	5.5	6	3.5	±	0.16	2.7	±	0.04	**
duration1.5_CMT_5	3.4	±	0.02	*ns*			3.4	±	0.01	3.2	±	0.11	*ns*
duration2_CMT_5	5.2	±	0.20	***			4.7	±	0.25	3.1	±	0.25	***
subproc1_CMT_5	6.4	±	0.11	*	5.5	6	6.9	±	0.14	5.9	±	0.14	***
total duration_CMT_5	16.4						17.2			11.7			

Table 4.9 (continued): Phenotypic performance of POP5 family for the onset of stage and duration. Parents mean ("Poli", "58-861") ± standard errors (SE) and Student's *t*-test significance differences (*t*-test) between them are given. Family genotypic variation with number of genotypes analyzed (gen.), average number of individuals per genotype (obs.), population means (μ), and *F*-test significance difference (*F*-test) among genotypes for POP5 grown in the environments are shown. The significance level of the statistical tests is indicated as: *ns* = non significant, * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$.

	VT 2010											
	Family phenotypic variation						Parent values					
Trait	**μ**	**±**	**SE**	***F*-test**	**obs.**	**gen.**	**Poli**	**±**	**SE**	**58-861**	**±**	**SE**	***t*-test**
bf_date0.5_DOY	82.2	±	0.13	***			80.0	±	0.93	86.6	±	0.60	***
bf_date1_DOY	87.4	±	0.13	***			85.7	±	1.17	93.0	±	0.77	***
bf_date1.5_DOY	92.1	±	0.16	***	4.5	156	89.7	±	1.24	97.8	±	0.66	***
bf_date2_DOY	97.8	±	0.16	***			97.6	±	1.02	102.4	±	0.56	***
bf_date2.5_DOY	102.9	±	0.14	***			105.4	±	0.74	105.8	±	0.61	*ns*
duration0.5_DOY	5.2	±	0.03	***			5.6	±	0.33	6.4	±	0.37	*ns*
duration1_DOY	4.7	±	0.04	***	4.5	156	4.1	±	0.15	4.8	±	0.24	*
duration1.5_DOY	5.7	±	0.05	***			7.9	±	0.28	4.6	±	0.47	***
duration2_DOY	5.1	±	0.06	***			7.7	±	0.36	3.4	±	0.64	***
subproc1_DOY	10.4	±	0.06	***	4.5	156	12.0	±	0.38	9.4	±	0.43	***
total duration_DOY	20.7						25.4			19.2			
bf_date0.5_CAT_{10}	77.1	±	0.19	***			74.3	±	1.28	84.0	±	0.83	***
bf_date1_CAT_{10}	84.5	±	0.21	***			81.0	±	1.42	91.3	±	0.67	***
bf_date1.5_CAT_{10}	90.6	±	0.18	***	4.5	156	87.9	±	1.42	96.0	±	0.61	***
bf_date2_CAT_{10}	96.5	±	0.15	***			96.7	±	1.22	100.8	±	0.61	**
bf_date2.5_CAT_{10}	101.5	±	0.16	***			104.8	±	1.30	104.6	±	1.20	*ns*
duration0.5_CAT_{10}	7.4	±	0.05	***			6.7	±	0.36	7.3	±	0.31	*ns*
duration1_CAT_{10}	6.1	±	0.05	***	4.5	156	7.0	±	0.41	4.8	±	0.15	***
duration1.5_CAT_{10}	5.9	±	0.05	***			8.8	±	0.46	4.7	±	0.20	***
duration2_CAT_{10}	5.0	±	0.08	***			8.1	±	0.52	3.8	±	0.78	***
subproc1_CAT_{10}	12.0	±	0.10	***	4.5	156	15.7	±	0.46	9.5	±	0.34	***
total duration_CAT_{10}	24.4						30.5			20.6			
bf_date0.5_CMT_5	341.7	±	0.09	***			340.9	±	0.92	345.3	±	0.71	***
bf_date1_CMT_5	346.5	±	0.13	***			344.8	±	1.20	352.0	±	0.82	***
bf_date1.5_CMT_5	351.4	±	0.16	***	4.5	156	349.3	±	1.15	357.2	±	0.67	***
bf_date2_CMT_5	357.2	±	0.15	***			358.4	±	0.79	361.5	±	0.41	**
bf_date2.5_CMT_5	361.5	±	0.14	***			363.3	±	0.58	364.3	±	0.31	*ns*
duration0.5_CMT_5	4.8	±	0.05	***			4.0	±	0.29	6.7	±	0.32	***
duration1_CMT_5	4.9	±	0.03	***	4.5	156	4.5	±	0.19	5.2	±	0.25	*
duration1.5_CMT_5	5.8	±	0.05	***			9.1	±	0.65	4.4	±	0.31	***
duration2_CMT_5	4.3	±	0.06	***			4.9	±	0.42	2.8	±	0.39	**
subproc1_CMT_5	10.7	±	0.07	***	4.5	156	13.6	±	0.71	9.6	±	0.46	***
total duration_CMT_5	19.7						22.5			19.1			

Table 4.9 (continued): Phenotypic performance of POP5 family for the onset of stage and duration. Parents mean ("Poli", "58-861") ± standard errors (SE) and Student's *t*-test significance differences (*t*-test) between them are given. Family genotypic variation with number of genotypes analyzed (gen.), average number of individuals per genotype (obs.), population means (μ), and *F*-test significance difference (*F*-test) among genotypes for POP5 grown in the environments are shown. The significance level of the statistical tests is indicated as: *ns* = non significant, * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$.

	VT 2012											
	Family phenotypic variation						Parent values					
Trait	**μ**	**±**	**SE**	***F*-test**	**obs.**	**gen.**	**Poli**	**±**	**SE**	**58-861**	**±**	**SE**	***t*-test**
bf_date0.5_DOY	75.9	±	0.09	***			73.2	±	0.33	80.9	±	1.01	***
bf_date1_DOY	81.6	±	0.11	***			77.8	±	0.45	85.7	±	0.84	***
bf_date1.5_DOY	86.0	±	0.12	***	4.0	124	82.2	±	0.58	89.4	±	0.70	***
bf_date2_DOY	90.4	±	0.12	***			87.7	±	0.46	93.4	±	0.68	***
bf_date2.5_DOY	93.3	±	0.10	***			93.0	±	0.00	95.7	±	0.58	**
duration0.5_DOY	5.7	±	0.04	*ns*			4.7	±	0.26	4.7	±	0.50	*ns*
duration1_DOY	4.5	±	0.03	*ns*	4.0	124	4.4	±	0.24	3.7	±	0.18	*
duration1.5_DOY	4.3	±	0.02	*ns*			5.4	±	0.24	4.0	±	0.07	***
duration2_DOY	3.0	±	0.05	*ns*			5.3	±	0.46	2.3	±	0.28	***
subproc1_DOY	8.8	±	0.05	*ns*	4.0	124	9.9	±	0.35	7.7	±	0.22	***
total duration_DOY	17.4						19.8			14.7			
bf_date0.5_CAT_{10}	81.1	±	0.15	***			77.6	±	0.31	91.7	±	2.89	**
bf_date1_CAT_{10}	92.5	±	0.33	***			84.2	±	0.67	105.8	±	2.71	***
bf_date1.5_CAT_{10}	107.8	±	0.39	***	4.0	115	94.2	±	2.17	117.4	±	1.98	***
bf_date2_CAT_{10}	120.1	±	0.37	***			113.2	±	1.22	128.2	±	1.71	***
bf_date2.5_CAT_{10}	128.1	±	0.39	***			126.6	±	0.00	136.1	±	2.05	**
duration0.5_CAT_{10}	11.4	±	0.19	***			6.6	±	0.42	14.1	±	1.98	**
duration1_CAT_{10}	15.4	±	0.19	*ns*	4.0	115	10.0	±	1.57	11.6	±	0.81	*ns*
duration1.5_CAT_{10}	12.3	±	0.11	*ns*			19.0	±	1.13	10.8	±	0.43	***
duration2_CAT_{10}	8.0	±	0.15	***			12.8	±	1.16	7.9	±	0.75	-
subproc1_CAT_{10}	27.7	±	0.21	*ns*	4.0	115	29.1	±	0.82	22.4	±	1.07	***
total duration_CAT_{10}	47.1						49.0			44.3			
bf_date0.5_CMT_5	446.4	±	0.05	***			444.8	±	0.36	448.6	±	0.41	***
bf_date1_CMT_5	449.0	±	0.05	***			447.4	±	0.25	450.5	±	0.26	***
bf_date1.5_CMT_5	450.7	±	0.04	***	4.0	124	449.4	±	0.20	451.8	±	0.20	***
bf_date2_CMT_5	452.2	±	0.03	***			451.4	±	0.14	453.1	±	0.22	***
bf_date2.5_CMT_5	453.2	±	0.01	*ns*			453.3	±	0.00	453.3	±	0.00	-
duration0.5_CMT_5	2.6	±	0.02	*ns*			2.6	±	0.19	1.9	±	0.17	**
duration1_CMT_5	1.7	±	0.01	*ns*	4.0	123	2.0	±	0.13	1.4	±	0.07	**
duration1.5_CMT_5	1.5	±	0.01	***			2.0	±	0.07	1.3	±	0.04	***
duration2_CMT_5	1.0	±	0.02	***			1.8	±	0.14	0.6	±	0.14	***
subproc1_CMT_5	3.2	±	0.02	*	3.8	115	4.0	±	0.19	2.7	±	0.08	***
total duration_CMT_5	6.9						8.5			4.7			

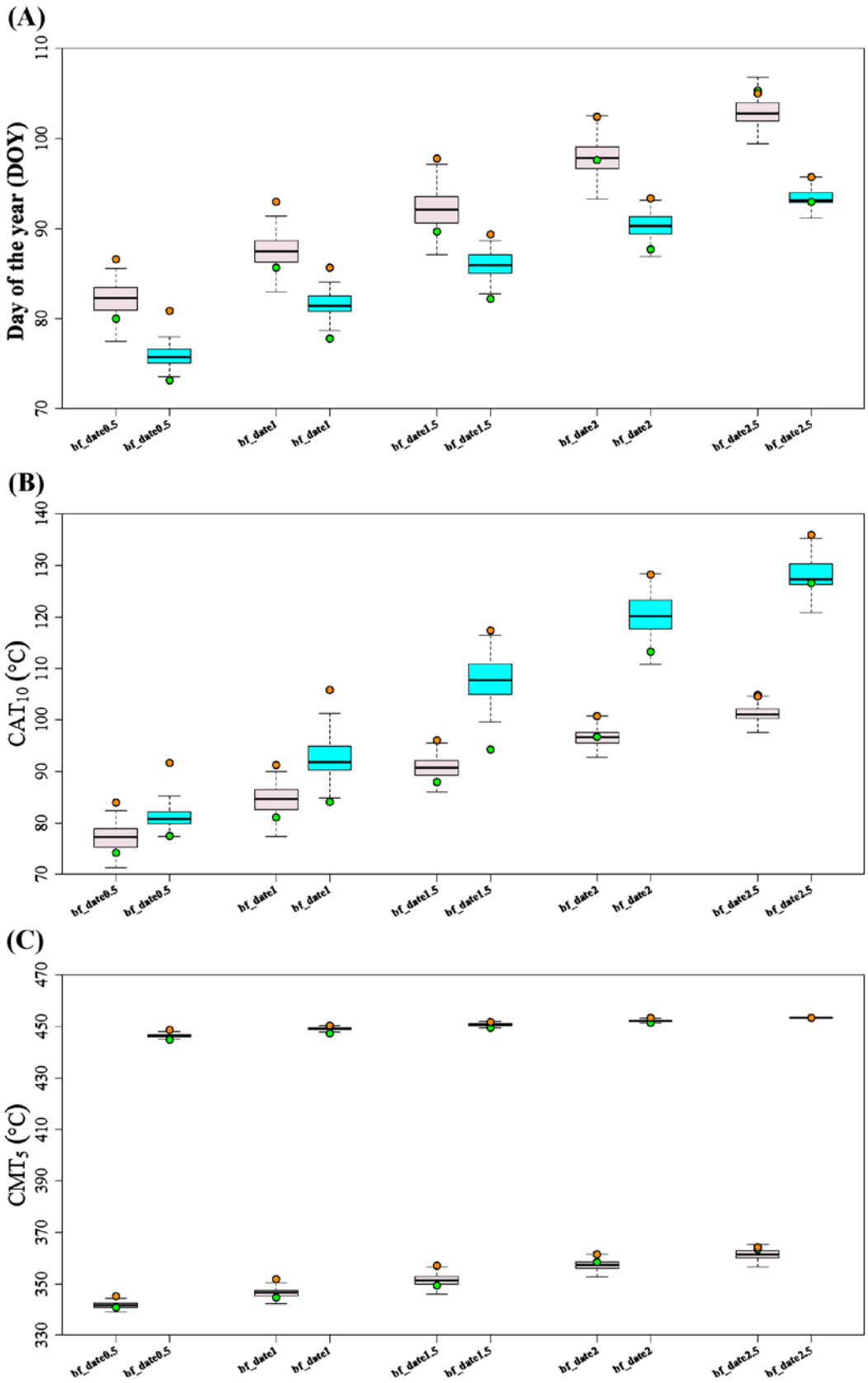

Figure 4.9: Genotypic variation from the bud swelling (bf_date0.5) to the complete leaf unfolding (bf_date2.5) observed in VT 2010 (lavender box) and VT 2012 (cyan box). Block-adjusted genotypic mean expressed as DOY (A), CAT_{10} (B) and CMT_5 (C) was used. The northern parent "58-861" (orange circle) and parent "Poli" (green circle) are also shown.

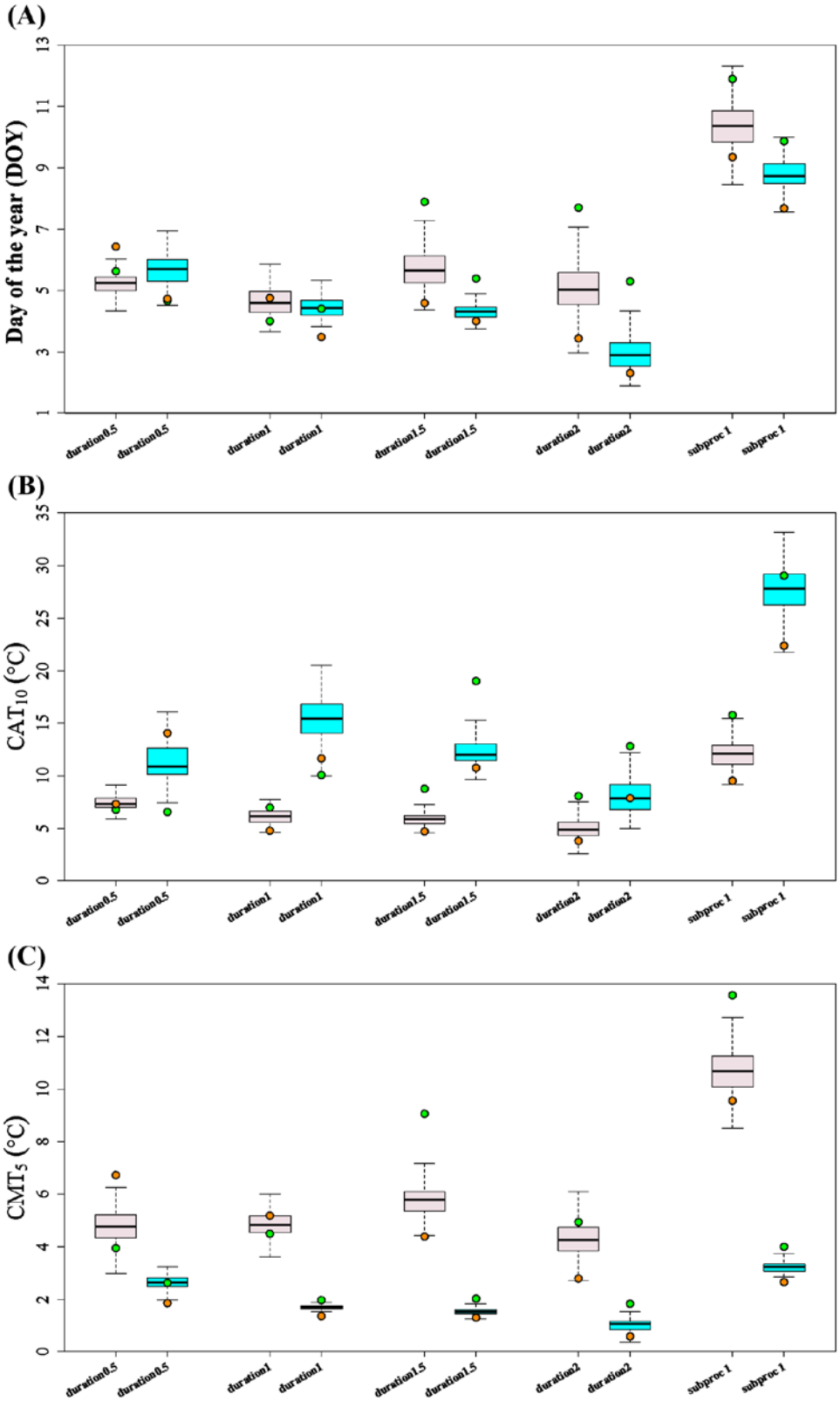

Figure 4.10: Genotypic variation of the duration of the different phases and of the subprocess observed in VT 2010 and VT 2012. Block-adjusted genotypic mean expressed as DOY (A), CAT_{10} (B) and CMT_5 (C) was used. The northern parent "58-861" (grey circle) and parent "Poli" (white circle) are also shown.

4.3.4. Within-site family variability and heritability

Genetic parameters (broad sense heritability and coefficient of variation) related to bud flush traits are shown. A considerable within year genetic variation for the full-sib family was observed in this study (Tab. 4.10), due to parents comes from widely contrast sites. Data showed higher values of genetic variability (CV_G) in VT 2010 than VT 2012 for all the traits expressed in DOY and CMT_5. Differently, for bf_date1.5, bf_date2, bf_date2.5 and duration of bf_date0.5 expressed in CAT_{10}, higher values were observed in VT 2012 than VT 2010. For environmental influence (CV_ε), a clear trend was not observed.

In VT 2010, for each onset-of-stage were observed very similar values of CV_G and CV_ε for the different climatic parameters, otherwise for duration traits were observed greater values of CV_ε than CV_G (often more the twice). In general, similar values of CV_G and CV_ε suggested the same magnitude of genetic and environmental variances, which was summarized in H_i^2 values closed around 0.50, between 0.29 (bf_date2.5 CAT_{10}) and 0.51 (bf_date1 CMT_5). Otherwise, the high values of CV_ε and the low values of CV_G pointed out higher influence of environmental condition on durations, and the subsequent very low values of heritability estimated (closed between 0.10 for duration0.5 and 0.46 for CMT_5 accumulation during bf_date0.5).

In VT 2012 different tendencies between CV_G and CV_ε were found, with greater values of residual variance than genetic variance observed for all phenological traits. About each onset-of-stage (expressed in DOY, CAT_{10} and CMT_5), CV_G values were approximately half of CV_ε values, with individual heritability values closed between 0.16 (bf_date0.5 DOY) and 0.29 (bf_date2 CAT_{10}). Very high values of CV_ε for duration (expressed in DOY, CAT_{10} and CMT_5) were recorded (closed between 11.11% for bf_date1 duration in CMT_5 and 35.85% for bf_date2 duration in CMT_5), with associated values of heritability between 0.01 (subprocess1 DOY) and 0.25 (CMT_5 accumulation during bf_date2).

Table 4.10: Family genetic variation for bud flush traits. Coefficients of Genetic (CV_G) and Residual (CV_e) Variation, individual (H_i^2) and genotypic (H_g^2) broad-sense heritability for the family are shown.

	CV 2004				MT 2004			
	Family genetic variation				Family genetic variation			
Trait	**CV_G**	**CV_ε**	**H^2_i**	**H^2_g**	**CV_G**	**CV_ε**	**H^2_i**	**H^2_g**
bf_date1.5_DOY	2.82	3.01	0.48	0.82	2.91	4.34	0.30	0.67
bf_date1.5_CAT_{10}	19.31	21.62	0.45	0.80	1.95	3.77	0.17	0.50
bf_date1.5_CMT_5	0.65	0.74	0.44	0.79	0.48	0.82	0.26	0.63
	VT 2010				**VT 2012**			
	Family genetic variation				Family genetic variation			
Trait	**CV_G**	**CV_ε**	**H^2_i**	**H^2_g**	**CV_G**	**CV_ε**	**H^2_i**	**H^2_g**
bf_date0.5_DOY	1.86	2.01	0.46	0.80	0.94	2.12	0.16	0.44
bf_date1_DOY	1.78	1.86	0.49	0.81	1.17	2.18	0.22	0.53
bf_date1.5_DOY	2.05	2.10	0.50	0.82	1.21	2.12	0.26	0.58
bf_date2_DOY	1.82	2.07	0.46	0.79	1.08	2.02	0.25	0.56
bf_date2.5_DOY	1.47	1.92	0.37	0.73	0.90	1.60	0.26	0.58
duration0.5_DOY	4.31	12.69	0.10	0.34	3.94	16.67	0.05	0.18
duration1_DOY	8.77	14.27	0.27	0.63	2.58	15.53	0.03	0.10
duration1.5_DOY	7.96	17.91	0.18	0.51	1.98	12.07	0.03	0.10
duration2_DOY	12.61	23.06	0.24	0.59	7.57	34.33	0.09	0.27
subproc1_DOY	5.66	12.00	0.19	0.52	1.51	12.31	0.01	0.06
bf_date0.5_CAT_{10}	2.86	3.02	0.47	0.80	1.31	3.07	0.19	0.48
bf_date1_CAT_{10}	2.85	2.97	0.50	0.82	2.77	5.37	0.22	0.52
bf_date1.5_CAT_{10}	2.19	2.49	0.47	0.80	2.75	5.48	0.23	0.54
bf_date2_CAT_{10}	1.70	2.00	0.45	0.78	2.52	4.26	0.29	0.62
bf_date2.5_CAT_{10}	1.59	2.54	0.29	0.65	1.96	3.30	0.26	0.58
duration0.5_CAT_{10}	5.60	14.57	0.12	0.40	13.12	25.36	0.20	0.50
duration1_CAT_{10}	8.52	15.32	0.25	0.60	4.31	25.06	0.03	0.12
duration1.5_CAT_{10}	8.41	17.15	0.28	0.64	3.74	19.65	0.03	0.11
duration2_CAT_{10}	14.01	35.13	0.18	0.49	12.47	32.52	0.16	0.43
subproc1_CAT_{10}	7.98	13.93	0.28	0.64	2.91	15.15	0.04	0.14
bf_date0.5_CMT_5	0.30	0.31	0.49	0.81	0.09	0.21	0.17	0.44
bf_date1_CMT_5	0.46	0.45	0.51	0.82	0.09	0.16	0.22	0.53
bf_date1.5_CMT_5	0.53	0.53	0.49	0.82	0.08	0.14	0.24	0.56
bf_date2_CMT_5	0.49	0.56	0.42	0.77	0.06	0.11	0.24	0.55
bf_date2.5_CMT_5	0.42	0.56	0.36	0.72	0.01	0.09	0.01	0.05
duration0.5_CMT_5	11.99	12.90	0.46	0.80	4.97	19.82	0.06	0.19
duration1_CMT_5	7.64	13.05	0.26	0.61	2.03	11.11	0.03	0.11
duration1.5_CMT_5	7.77	16.78	0.17	0.48	5.19	13.23	0.14	0.39
duration2_CMT_5	11.98	26.61	0.17	0.48	18.53	35.85	0.25	0.57
subproc1_CMT_5	5.48	12.72	0.16	0.47	3.43	11.23	0.09	0.29

4.3.5. Analysis of variance between environments and genotype stability

Genotype and environment effects were significant for all the analyzed data, with preponderant role of environment to define total variance observed in the trials (Tab. 4.11). In VT site, for the onset-of-stage expressed in DOY, it was possible to observed few variance between stages for σ^2_E/σ^2_P (closed around a mean value of 80%), with invariable values of σ^2_G/σ^2_P (closed around a mean value of 5%), that suggested a steady difference of day for stages between the years (Fig. 4.9 A). Moreover, closed values (between 6% and 14 %) of residual variance component ($\sigma^2_\varepsilon/\sigma^2_P$) (i.e. it explains variance components due to unknown effects) remarked the previous observed behavior. Significant G×E interaction was observed for bf_date1, bf_date1.5 and bf_date2, whit very low values of relative variance components and high values of significant Spearman's rank coefficient (greater than 0.55) suggested a lack of random changes of ranks.

Otherwise, for the same traits analyzed with CAT_{10} were observed a rising of σ^2_E/σ^2_P with a decrement value for σ^2_G/σ^2_P, in agreement with process trends observed in the years (Fig. 4.9 B). For bf_date0.5 were observed values of σ^2_E/σ^2_P = 47.13% and σ^2_G/σ^2_P = 16.94%, that underlined very similar values of CAT_{10} accounting in VT 2010 and VT 2012 (77.1°C and 81°C, respectively). Not significant G×E interaction was observed for all the five stages.

An extreme distribution of relative variance components were observed for CMT_5 used to describe the onset-of-stage, with the total variance explained by environment effect (99.97%), due to greater variance between years (more than 100°C of difference), rather than genotype variance observed in each stage (Fig. 4.9 C). The other sources of variance were closed to 0, with the $\sigma^2_{G\times E}/\sigma^2_P$ often too low to be quantified (bf_date0.5, bf_date1 and bf_date2.5)

Concerning the duration of stages, a general preponderance of σ^2_E/σ^2_P and $\sigma^2_\varepsilon/\sigma^2_P$ were observed, without a clear trends through the stages. For these traits, was often observed a significant G×E interaction, with considerable values (9.21% and 4.09%) for the duration of bud breaking (duration1) and for onset of leaf elongation (duration1.5).

In CV 2004 vs. MT 2004 it was possible observed relevant values of $\sigma^2_{G\times E}/\sigma^2_P$ (7.57%) for bf_date1.5 expressed in DOY, with not negligible values of σ^2_G/σ^2_P (9.43%). Significant values of Spearman's coefficient ρ (0.37) suggested a pattern of genotype rank distribution between the two sites.

Relative ecovalence showed the same trends observed for biomass traits, with greater W^2_2 (average of 59% for VT 2012) than W^2_1 (average of 41% for VT 2010). In CV 2004 vs. MT 2004, equal

partition of ecovalence occurred between the two environments. Furthermore, like observed for biomass traits only a few proportion on genotype (closed values between 10% and 17%), corresponding to 16-17 genotypes, accounted at least 50% of total relative ecovalence (Tab. 4.11, Fig. 4.11).

Table 4.11: Relative importance of genetic (σ^2_G), environment (σ^2_E), genotype by environment ($\sigma^2_{G\times E}$), and residual (σ^2_ε) effects in the phenotypic variation (σ^2_P), with their statistic significance. Spearman's rank correlation coefficient (ρ) with his statistic significance (*P*-value) is shown. Distributed relative ecovalence between first (W^2_1) and second (W^2_2) environment is shown. Percentage of genotypes accounting 50% of the total G × E observed ($W^2_{50\%}$) and were below ecovalence mean value ($<\mu_w$) are shown. Note: *ns* = non significant, * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$, - = too low to be quantified.

		Variance components (%)							Spearman		Relative Ecovalance (%)			
Env.	**Trait**	σ^2_E/σ^2_P		σ^2_G/σ^2_P		$\sigma^2_{G\times E}/\sigma^2_P$		$\sigma^2_\varepsilon/\sigma^2_P$	**ρ**	***P*-value**	W^2_1	W^2_2	$W^2_{50\%}$	$<\mu_w$
CV 2004 vs. MT 2004	bf_date1.5_DOY	52.74	***	9.43	***	7.57	***	30.26	0.37	***	50.7	49.3	13.9	68.1
	bf_date1.5_CAT_{10}	99.66	***	0.06	***	0.05	***	0.22	0.40	***	51.5	48.5	14.6	64.6
	bf_date1.5_CMT_5	99.97	***	-	***	-	***	0.02	0.27	***				
VT 2010 vs. VT 2012	bf_date0.5_DOY	84.19	***	4.91	***	0.25	*ns*	10.65	0.71	***				
	bf_date1_DOY	80.25	***	6.12	***	0.90	*	12.73	0.64	***	41.4	58.6	15.0	64.2
	bf_date1.5_DOY	77.73	***	6.85	***	1.30	*	14.13	0.62	***	41.6	58.4	16.7	64.2
	bf_date2_DOY	84.23	***	4.26	***	0.79	*	10.73	0.56	***	42.2	57.8	13.3	70.0
	bf_date2.5_DOY	90.79	***	2.69	***	0.43	*ns*	6.09	0.47	***				
	duration0.5_DOY	13.71	***	2.66	**	3.60	*ns*	80.03	0.06	*ns*				
	duration1_DOY	5.29	***	6.22	***	9.21	**	79.28	0.28	**	41.3	58.7	10.0	70.8
	duration1.5_DOY	60.72	***	1.25	***	4.09	**	33.93	0.27	**	41.3	58.7	10.0	75.0
	duration2_DOY	66.87	***	4.74	***	1.12	*ns*	27.27	0.34	**				
	subproc1_DOY	48.03	***	2.50	***	3.86	*	45.60	0.27	**	41.4	58.6	10.8	71.7
	bf_date0.5_CAT_{10}	47.13	***	16.94	***	1.96	*ns*	33.97	0.70	***				
	bf_date1_CAT_{10}	61.93	***	12.13	***	-	*ns*	25.94	0.68	***				
	bf_date1.5_CAT_{10}	86.34	***	3.23	***	-	*ns*	10.43	0.59	***				
	bf_date2_CAT_{10}	94.51	***	1.45	***	0.04	*ns*	4.00	0.60	***				
	bf_date2.5_CAT_{10}	96.35	***	0.80	***	0.07	*ns*	2.78	0.47	***				
	duration0.5_CAT_{10}	62.29	***	0.85	***	5.60	***	31.26	-0.02	*ns*	41.5	58.5	15.3	66.7
	duration1_CAT_{10}	88.23	***	-	***	1.86	***	9.92	-0.08	*ns*	40.3	59.7	13.5	65.8
	duration1.5_CAT_{10}	90.47	***	0.83	***	0.91	**	7.79	0.23	*	40.4	59.6	13.5	68.5
	duration2_CAT_{10}	52.40	***	3.49	***	3.64	**	40.47	0.20	*	39.0	61.0	9.9	72.1
	subproc1_CAT_{10}	93.40	***	0.10	***	1.09	***	5.41	0.08	*ns*	40.0	60.0	10.8	65.8
	bf_date0.5_CMT_5	99.97	***	0.01	***	-	*	0.02	0.67	***				
	bf_date1_CMT_5	99.97	***	0.01	***	-	*	0.02	0.68	***				
	bf_date1.5_CMT_5	99.97	***	0.01	***	-	*ns*	0.02	0.65	***				
	bf_date2_CMT_5	99.97	***	0.01	***	-	*ns*	0.02	0.58	***				
	bf_date2.5_CMT_5	99.97	***	-	***	-	***	0.02	0.10	*ns*				
	duration0.5_CMT_5	83.01	***	-	***	4.87	***	12.11	-0.11	*ns*	41.9	58.1	13.4	67.2
	duration1_CMT_5	97.43	***	-	***	0.33	***	2.24	0.04	*ns*	41.9	58.1	10.1	70.6
	duration1.5_CMT_5	97.64	***	0.21	***	0.17	**	1.98	0.40	***	41.4	58.6	12.6	68.1
	duration2_CMT_5	88.55	***	1.67	***	0.41	*ns*	9.37	0.43	***				
	subproc1_CMT_5	98.03	***	0.07	***	0.19	**	1.71	0.20	*	40.3	59.7	12.6	66.7

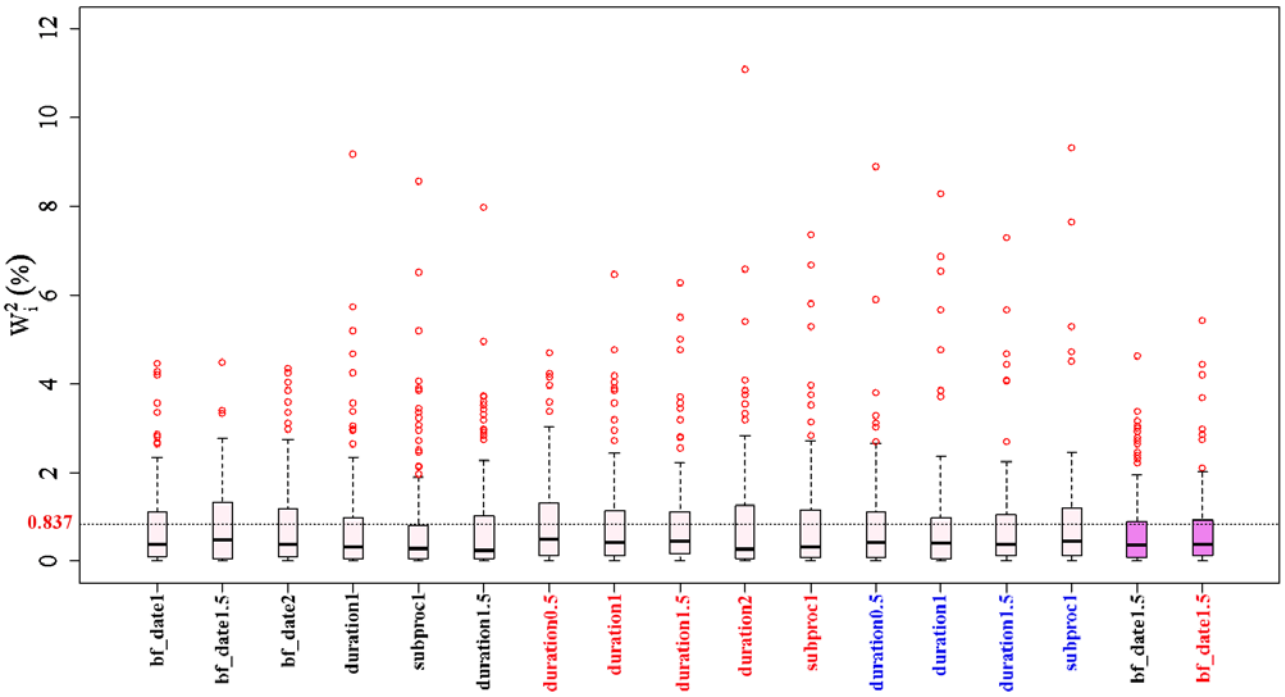

Figure 4.11: Genotype distribution of the relative genotype ecovalence (W^2_i) for the different bud flush traits with significant G×E effect. Results for VT 2010 vs. VT 2012 (lavender) and CV 2004 vs. MT 2004 (violet) are shown. Dotted horizontal line represent overall mean ecovalence value among environments. Different colors of labels indicate DOY (black), CAT_{10} (red) and CMT_5 (blue) parameters.

4.4. Definition of the most descriptive traits based on phenotypic variation

Phenotypic correlations among selected traits of particular interest for biomass and bud flush are shown. A heat map representation was used to emphasize the stronger and the weaker relationships between traits. Correlation values with statistical test significances are shown in Appendix 1.

For all the environments were observed low values of correlation (closed between 0.30 and -0.16) among traits related to biomass (Syll1, Height2, Circum2, TotalDM) and traits related to bud flush timing (bf_date1.5, bf_date2, bf_date2, subproc1) (Fig. 4.12). Differently, high correlations were observed inside the same kind of traits. Circumference and total dry mass showed the highest values (more than 0.90) and in CV 2004 and MT 2004 the number of sylleptic branches showed medium correlation with the other biometric traits (closed between 0.30 and 0.40). In VT 2010, medium correlation (0.52) between onset-of-stage of bf_date2 (DOY) and subproc1 duration (DOY) were observed. Differently, in VT 2012 low values (0.25) between the same traits were observed. For both the years 2010 and 2012, greater values of correlation between bf_date2 and subproc1, compare to correlation between bf_date1.5 and subproc1 were observed. These evidences suggested a most vulnerability to low temperature by second part of bud flush process than the first part.

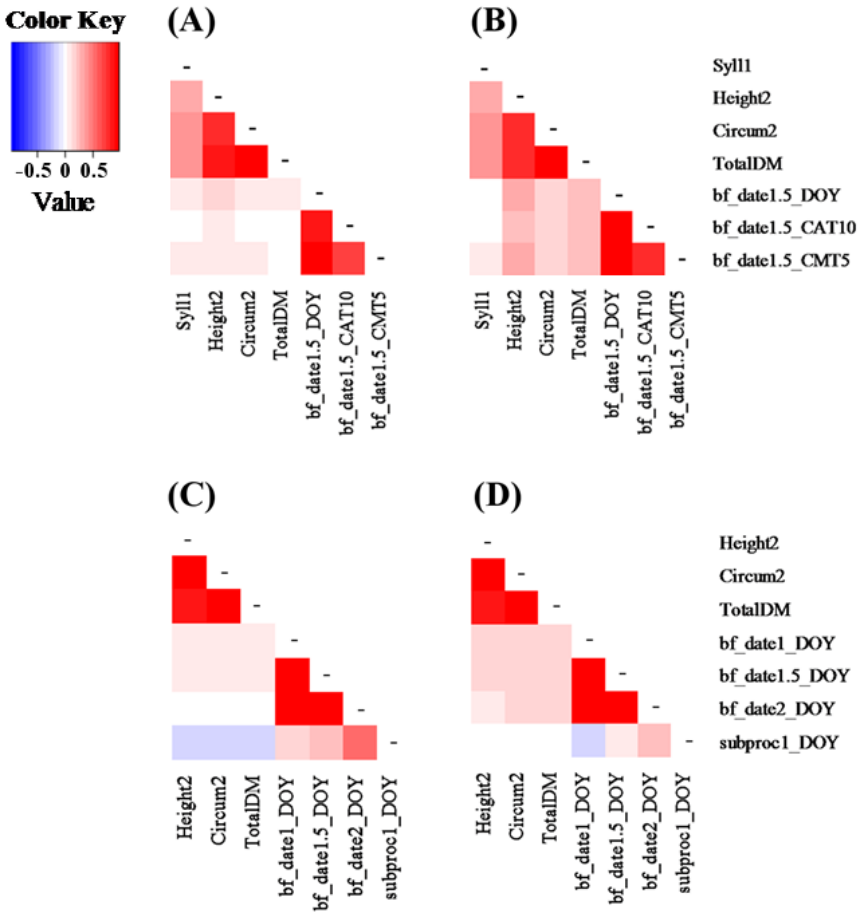

Figure 4.12: Pearson's phenotypic correlations between a selection of biomass and bud flush traits, based on genotypic means in the four environments: (A) CV 2004, (B) MT 2004, (C) VT 2010 and (D) VT 2012. Values change from -1 (dark blue) to +1 (dark red), by way of 0 value (white).

In the two experimental years (VT 2010 and VT 2012) the onset-of-stage of each phase resulted positively highly correlated with the other, especially between contiguous phases (more than 0.67) (Fig. 4.13). Overall, highly negative or weak correlation between phases and their duration expressed in CAT_{10} (Fig. 4.13 A and B) and CMT_5 (Fig. 4.13 C and D) were found. In the specific, in VT 2010 a clear pattern between date of onset-of-stages and durations expressed in CAT_{10} (Fig. 4.13 A) were observed, where negative values related to duration1 and duration1.5 pointed out drop in temperature occurred in the middle part of process. Otherwise for VT 2012, marked by more stable temperature trend, weaker correlations were observed among onset-of-stage and durations (Fig. 4.13 B). Concerning cold accumulation (CMT_5), in VT 2010 were observed strong positive correlation among duration1 and successive stages (closed between 0.62 and 0.83), and medium-low correlations among duration1.5 and subsequent phases (closed between -0.33 and 0.26) (Fig. 4.13 C). For VT 2012, a well-defined pattern among traits was observed, with negative correlations (from 0 to -0.86) which reflect the lack of CMT_5 increment during the process.

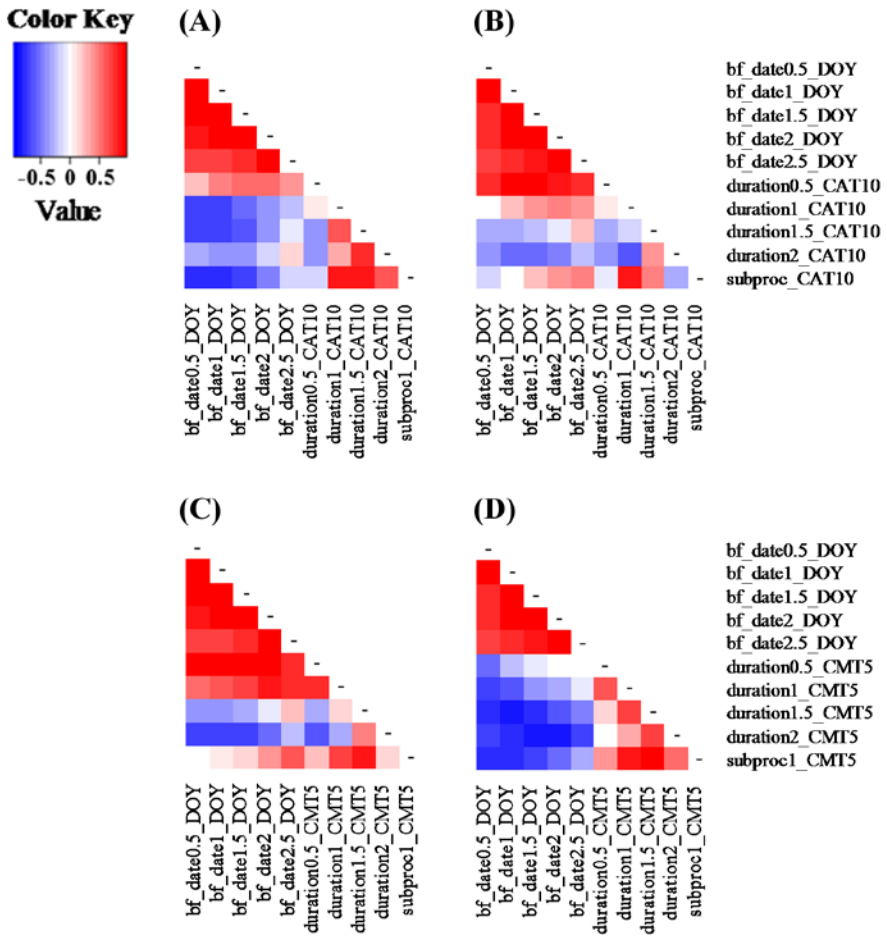

Figure 4.13: Pearson's phenotypic correlations among onset-of-stage traits (DOY) and their duration (CAT_{10} and CMT_5) based on genotypic means for VT 2010 (A and C) and VT 2012 (B and D). Values change from -1 (dark blue) to +1 (dark red), by way of 0 value (white).

Principal component analysis using the five onset-of-stage traits and the subprocess was performed in order to avoid needless information such as high correlated or redundant traits (CAT_{10} and CMT_5) in genetic analyses. This analysis allowed to identifying the most discriminative traits in term of phenotypic variation in the full-sib family. The different duration traits were not included because wholly represented by the duration of the subprocess. In both the years (VT 2010 and VT 2012), the phenotypic variation was widely explained by two principal components: Princ. Comp. 1 representing 79.7% in VT 2010 of the phenotypic variation, while 72.9% in VT 2012. Princ. Comp. 2 accounts for about 17.5% in VT 2010 and 22.1% in VT 2012 of the same variance (Fig. 4.14). Very similar values were observed for stages analyzed with CMT_5 parameter (data not shown). Both environments showed that the onset-of-stage traits, really important for a deep physiological dissection of leaves development, had a main contribute along the first principal component.

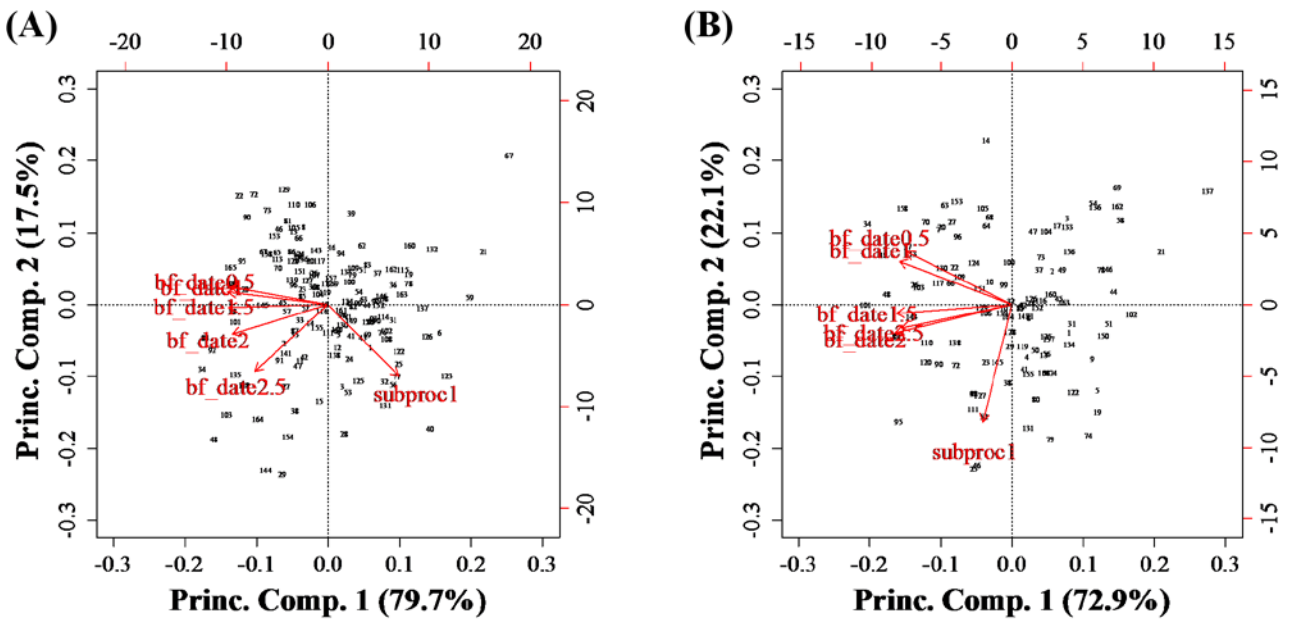

Figure 4.14: Principal component analysis (PCA) performed on the five onset-of-stage and the subprocess duration expressed in CAT_{10}, for VT 2010 (A) and VT 2012 (B). Genotypic adjusted means values were used.

In VT 2010, phases 0.5, 1, 1.5 and 2 had the major effect (-0.442, -0.452, -0.448 and -0.432 respectively) while duration of subproc1 for CAT_{10} had a minor opposing effect (0.319) on the Princ. Comp. 1. Duration of subproc1 and bf_date2.5 contributed most to Princ. Comp. 2 (-0.685 and -0.637), with minor effect for bf_date1.5 (-0.030). In VT 2012, bf_date1.5 and bf_date2 had the major effect on the Princ. Comp. 1 (-0.457 and -0.463). The duration of subprocess showed the major effect for Princ. Comp. 2 (-0.835). This analysis allowed selection of common traits in the two years, consisting in the leaf initiation stages (bf_date1, bf_date1.5, bf_date2) and their total duration (subproc1), to evaluate overlapping genomic region for genetic architecture of bud flush process.

For traits concerning biomass, the PCA analysis did not provide useful information to discriminate more patterns of traits (data not shown) that simplify phenotypic variance, so all biometric (Syll1, Height1, Height2, Circum2) and productive traits (TotalDM, StemDM and BranchDM) were studied in the QTLs analysis.

4.5. Number of QTLs and percentage of variance explained

4.5.1. Aboveground biomass

QTL analyses, performed on different environments and/or in different years, provide information on the QTLs stability and on the factors influencing the QTLs detection. On the maternal map "58-861" (Tab. 4.12, Fig. 4.15), 16, 15 and 13 QTLs were found in three multi-environments analyses (CV 2004 vs. MT 2004, SAV 2010 vs. VT 2010, and VT 2010 vs. VT 2012). A total number of 44 QTLs on 8 genomic regions were found. The phenotypic variance explained (PVE) by these QTLs ranged from 0.1% to 27.9%, with an average value of 6.4% across all individual QTLs (Tab. 4.12). QTLs detected on linkage group (LG) VI in VT 2010 and VT 2012 presented the highest PVE values, closed between 20.3% and 27.9%. Four QTL had significant difference of additive effect between CV and MT sites and five QTL had significant differences in VT site between the year 2010 and 2012 (Tab. 4.12). Four QTLs were found on the LG VI in CV 2004 and MT 2004, with PVE values greater than 8% in the former one. A large number of QTLs were found on LG XIX, with three QTLs detected for Height1, one for each multi-environment analysis and two QTLs found for Height2 on the same linkage group.

On the paternal map "Poli" (Tab. 4.13, Fig. 4.16), a total number of 16, 18 and 7 QTLs were found in the three multi-environments analyses presented above. In total, 41 QTLs were found on 11 different genomic regions. The phenotypic variance explained by single QTL range from 0.2% to 14.1%. A large number of QTLs were found on LG IIa, IIb and III. These QTLs, on LG III, also presented the greater values of PVE in SAV 2010, from 11.2% to 14.1%. Only one QTL had significant different effect between CV and MT sites in year 2004 (Tab. 4.13).

Four common markers were found between maternal and paternal maps on four different linkage groups. These four markers are microsatellites, also known as simple sequence repeats (SSRs) or short tandem repeats (STRs).

- ORPM_127 on LG IV, mapped near (less than 20 cM of distance) the peaks of 4 QTLs detected on maternal map,
- PMGC_2839 on LG V, mapped near (less than 10 cM of distance) the peaks of 2 QTLs detected on paternal map,
- PMGC_2423 on LG VI, overlapped on the peaks of 2 QTLs detected on paternal map,
- PMGC_520 on LG XV, overlapped on the peaks of 2 QTLs detected on paternal map.

Table 4.12: QTLs detected in the maternal, "58-861", map for traits related to aboveground biomass production. For each QTL, the linkage groups (LG), the LOD (logarithm of the odds), the position of peak (in cM), the 95% confidence interval (95% CI) in cM, the percentage of phenotypic variance explained (PVE) at each environment are shown. LG refers to the map in figure 4.15. Statistical significance (P-value) of different weight of the two environments is given (ns = non significant, * = P ≤ 0.05, ** = P ≤ 0.01, *** = P ≤ 0.001).

CV 2004 vs. MT 2004										
Trait	**LG**	**LOD**	**Peak**	**95% CI**		***P-value***	**CV 2004**		**MT 2004**	
							Effect	**PVE(%)**	**Effect**	**PVE(%)**
Syll1	I	3.4	53.6	0.0	175.0	*ns*	-3.5	6.0	-4.8	5.7
Height1	III	3.2	76.8	32.2	113.3	*	15.5	8.8	-1.8	0.1
Syll1	V	4.0	0.0	0.0	17.4	*ns*	3.0	4.4	7.3	13.3
Circum2	VI	3.7	186.8	68.3	209.8	*ns*	0.8	3.1	0.9	11.1
StemDM	VI	2.9	194.7	54.4	209.8	*ns*	156.0	3.2	195.6	8.7
BranchDM	VI	4.0	183.6	67.2	209.8	*ns*	162.1	7.3	177.9	8.5
TotalDM	VI	2.9	187.9	17.0	209.8	*ns*	231.0	2.5	384.4	9.0
Syll1	XI	3.1	91.0	62.2	91.0	*ns*	-4.7	10.3	-2.5	1.6
Syll1	XII	3.3	23.1	0.0	60.0	*ns*	1.9	1.6	5.7	8.2
Circum2	XII	2.9	0.0	0.0	51.5	*ns*	1.2	7.5	0.4	1.9
TotalDM	XII	2.3	0.0	0.0	62.2	*ns*	351.0	5.7	177.1	2.0
Height1	XV	2.7	58.3	37.9	68.9	**	5.4	1.1	-17.8	9.2
Height2	XV	2.0	48.1	17.5	68.9	**	8.9	1.1	-17.1	6.0
BranchDM	XVIII	2.7	84.5	48.8	84.5	*ns*	-160.0	7.1	-97.7	2.6
Height1	XIX	3.5	92.7	40.3	92.7	*ns*	16.8	10.4	10.5	3.2
Height2	XIX	2.3	92.7	14.9	92.7	*	40.3	9.0	4.5	0.4
Mean								5.6		5.7
SAV 2010 vs. VT 2010										
Trait	**LG**	**LOD**	**Peak**	**95% CI**		***P-value***	**SAV 2010**		**VT 2010**	
							Effect	**PVE(%)**	**Effect**	**PVE(%)**
StemDM	I	2.9	267.0	0.0	306.2	*ns*	-408.4	3.0	-591.4	5.1
Height2	IV	2.7	24.5	0.0	85.4	*ns*	11.2	2.2	27.5	5.7
Circum2	IV	2.6	24.5	0.0	80.5	*ns*	0.8	2.0	1.5	5.8
BranchDM	IV	2.7	24.5	0.0	72.5	*ns*	176.6	2.4	282.4	6.0
TotalDM	IV	3.0	24.5	0.0	62.7	*ns*	552.8	2.5	975.5	6.8
Height2	V	2.5	12.6	0.0	40.8	*ns*	-20.5	7.1	-20.5	3.4
Circum2	V	3.3	10.0	0.0	33.6	*ns*	-1.9	9.8	-1.4	4.8
StemDM	V	2.7	12.6	0.0	39.6	*ns*	-598.2	6.4	-547.5	4.4
BranchDM	V	2.7	8.9	0.0	32.7	*ns*	-327.6	8.1	-214.8	3.6
TotalDM	V	2.8	9.4	0.0	33.2	*ns*	-1040.0	8.5	-709.2	3.7
Height1	IX	3.6	109.0	66.5	139.7	*ns*	-15.5	6.5	-15.3	4.6
Height2	XII	2.6	63.1	14.1	89.2	*ns*	-16.5	4.6	-17.9	2.6
Height1	XVII	3.2	63.4	28.1	87.2	*ns*	13.9	5.4	16.0	4.9
Height1	XIX	3.5	92.7	40.8	92.7	*ns*	13.1	4.8	19.7	7.5
StemDM	XIX	2.4	91.8	45.6	92.7	*ns*	505.8	4.6	527.0	4.1
Mean								5.2		4.9

Tab. 4.12 (continued): QTLs detected in the maternal, "58-861", map for traits related to aboveground biomass production. For each QTL, the linkage groups (LG), the LOD (logarithm of the odds), the position of peak (in cM), the 95% confidence interval (95% CI) in cM, the percentage of phenotypic variance explained (PVE) at each environment are shown. LG refers to the map in figure 4.15. Statistical significance (P-value) of different weight of the two environments is given (ns = non significant, * = P ≤ 0.05, ** = P ≤ 0.01, *** = P ≤ 0.001).

VT 2010 vs. VT 2012										
Trait	**LG**	**LOD**	**Peak**	**95% CI**		***P-value***	**VT 2010**		**VT 2012**	
							Effect	**PVE(%)**	**Effect**	**PVE(%)**
Height2	VI	5.5	40.6	15.2	73.5	*ns*	-23.3	4.2	-50.9	20.3
Circum2	VI	6.4	36.0	9.2	67.7	*	-12.0	3.7	-35.6	26.6
StemDM	VI	6.4	34.5	6.5	66.1	*	-453.0	2.7	-1454.8	27.5
BranchDM	VI	6.3	34.2	7.0	68.0	**	236.0	4.2	-800.0	27.4
TotalDM	VI	6.5	34.2	8.3	64.4	**	-804.7	4.7	-2211.5	27.9
Height1	IX	3.3	6.2	0.0	95.0	*	2.7	0.1	-23.9	13.9
Height2	XV	2.5	35.9	15.1	57.5	*ns*	4.6	0.2	21.2	8.9
Height1	XIX	2.7	92.7	20.4	92.7	*ns*	20.3	7.8	11.8	3.4
Height2	XIX	2.5	92.7	8.4	92.7	*ns*	30.7	7.3	20.3	3.2
Circum2	XIX	2.6	92.7	6.5	92.7	*ns*	14.7	5.1	15.7	5.7
StemDM	XIX	2.4	92.7	6.4	92.7	*ns*	613.9	5.4	617.2	5
BranchDM	XIX	2.3	92.7	5.8	92.7	*ns*	231.2	4.1	364.3	5.6
TotalDM	XIX	2.2	92.7	2.9	92.7	*ns*	748.4	4.1	959.1	5.2
Mean								4.1		13.8

Table 4.13: QTLs detected in the paternal, "Poli", map for traits related to aboveground biomass production. For each QTL, the linkage groups (LG), the LOD (logarithm of the odds), the position of peak (in cM), the 95% confidence interval (95% CI) in cM, the percentage of phenotypic variance explained (PVE) at each environment are shown. LG refers to the map in figure 4.16. Statistical significance (P-value) of different weight of the two environments is given (ns = non significant, * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$).

CV 2004 vs. MT 2004										
Trait	**LG**	**LOD**	**Peak**	**95% CI**		***P-value***	**CV 2004**		**MT 2004**	
							Effect	**PVE(%)**	**Effect**	**PVE(%)**
BranchDM	Ib	3.2	25.8	0.0	71.4	*ns*	-142.7	6.4	-127.9	4.4
Circum2	IIb	4.1	7.9	0.0	39.5	*ns*	1.1	6.2	0.8	8.4
StemDM	IIb	3.7	2.9	0.0	31.2	*ns*	179.9	4.0	195.5	8.4
BranchDM	IIb	4.8	0.0	0.0	22.3	*ns*	158.2	7.1	184.2	9.1
TotalDM	IIb	3.7	0.7	0.0	32.6	*ns*	282.0	3.8	369.7	8.4
Height2	III	2.7	91.9	20.9	156.1	*	-23.7	7.9	-3.0	0.2
StemDM	III	3.5	17.9	0.0	125.4	*ns*	-283.1	9.8	-113.2	2.8
Sylleptic1	IVb	3.1	66.4	24.7	95.6	*ns*	4.5	9.6	1.8	0.8
Circum2	VIa	3.1	120.1	69.8	131.6	*ns*	-1.1	6.5	-0.4	1.5
TotalDM	VIa	2.5	122.9	61.3	131.6	*ns*	-356.8	5.9	-168.4	1.8
Circum2	XIV	2.8	38.0	1.7	70.7	*ns*	0.8	3.5	0.8	7.4
StemDM	XIV	3.2	50.2	7.5	75.7	*ns*	226.9	6.6	313.5	1.8
Height2	XV	3.0	72.7	17.9	80.2	*ns*	-13.3	2.6	-20.1	8.1
Sylleptic1	XVII	2.9	172.0	109.6	177.2	*ns*	-4.5	9.8	-3.7	3.3
Height2	XVII	2.9	164.7	106.1	177.2	*ns*	-21.0	6.4	-16.1	5.3
Circum2	XVII	4.5	162.9	74.6	177.2	*ns*	-1.5	13.4	-0.5	3.5
Mean								6.8		4.7
SAV 2010 vs. VT 2010										
Trait	**LG**	**LOD**	**Peak**	**95% CI**		***P-value***	**SAV 2010**		**VT 2010**	
							Effect	**PVE(%)**	**Effect**	**PVE(%)**
Height1	IIa	3.5	108.4	65.0	148.0	*ns*	-15.5	6.7	-15.5	4.6
Height2	IIa	5.2	107.2	76.8	136.1	*ns*	-26.7	12.5	-22.3	3.9
Circum2	IIa	4.0	106.3	68.1	140.0	*ns*	-1.8	9.0	-1.1	3.4
StemDM	IIa	5.2	108.5	72.9	139.7	*ns*	-816.5	11.7	-538.3	4.1
BranchDM	IIa	4.3	109.5	85.3	137.5	*ns*	-365.9	10.1	-207.3	3.2
TotalDM	IIa	3.4	107.0	56.4	151.1	*ns*	-978.4	7.8	-615.8	2.8
Height1	III	4.0	26.0	0.0	100.3	*ns*	-20.1	11.2	-13.2	3.3
Height2	III	4.6	16.3	0.0	74.1	*ns*	-28.2	14.1	-7.9	0.5
Circum2	III	4.9	12.3	0.0	98.4	*ns*	-2.0	12.2	-0.9	1.9
StemDM	III	5.5	13.9	0.0	77.1	*ns*	-837.2	12.5	-415.7	2.4
BranchDM	III	5.8	12.3	0.0	79.8	*ns*	-409.8	13.1	-202.9	3.0
TotalDM	III	5.0	12.3	0.0	84.3	*ns*	-1221.1	12.3	-556.1	2.2
Circum2	Va	2.5	124.9	73.4	143.7	*ns*	1.1	3.6	1.3	4.2
TotalDM	Va	2.7	124.0	84.5	143.7	*ns*	664.7	3.8	821.6	4.6
Height2	XII	2.5	99.7	52.3	99.7	*ns*	-8.5	1.3	-30.2	6.8
StemDM	XIV	4.0	83.5	70.1	94.4	*ns*	521.9	5.1	593.0	4.7
BranchDM	XIV	3.4	82.4	69.5	92.8	*ns*	233.5	4.5	262.7	4.8
StemDM	XVIII	2.5	75.0	29.1	75.0	*ns*	385.1	2.8	494.7	3.3
Mean								8.3		3.6

Table 4.13 (continued): QTLs detected in the paternal, “Poli”, map for traits related to aboveground biomass production. For each QTL, the linkage groups (LG), the LOD (logarithm of the odds), the position of peak (in cM), the 95% confidence interval (95% CI) in cM, the percentage of phenotypic variance explained (PVE) at each environment are shown. LG refers to the map in figure 4.16. Statistical significance (P-value) of different weight of the two environments is given (ns = non significant, * = P ≤ 0.05, ** = P ≤ 0.01, *** = P ≤ 0.001).

VT 2010 vs. VT 2012										
Trait	**LG**	**LOD**	**Peak**	**95% CI**		***P-value***	**VT 2010**		**VT 2012**	
							Effect	**PVE(%)**	**Effect**	**PVE(%)**
Height1	Va	2.4	26.4	0.0	143.7	*ns*	8.6	1.4	18.3	8.5
Height2	XI	2.5	16.7	0.0	72.6	*ns*	-10.1	0.8	-38.8	12.1
Circum2	XI	3.5	20.6	0.0	63.6	*ns*	-8.4	1.8	-24.5	12.1
StemDM	XI	2.4	24.6	0.0	77.6	*ns*	-320.3	1.4	-732.0	7.2
Circum2	XIII	2.5	0.0	0.0	54.2	*ns*	10.6	2.8	17.2	6
BranchDM	XIII	2.3	34.5	0.0	55.9	*ns*	13.8	2.4	381.0	5.8
TotalDM	XIII	2.3	34.5	0.0	66.2	*ns*	614.0	2.7	1006.0	5.8
Mean								1.9		8.2

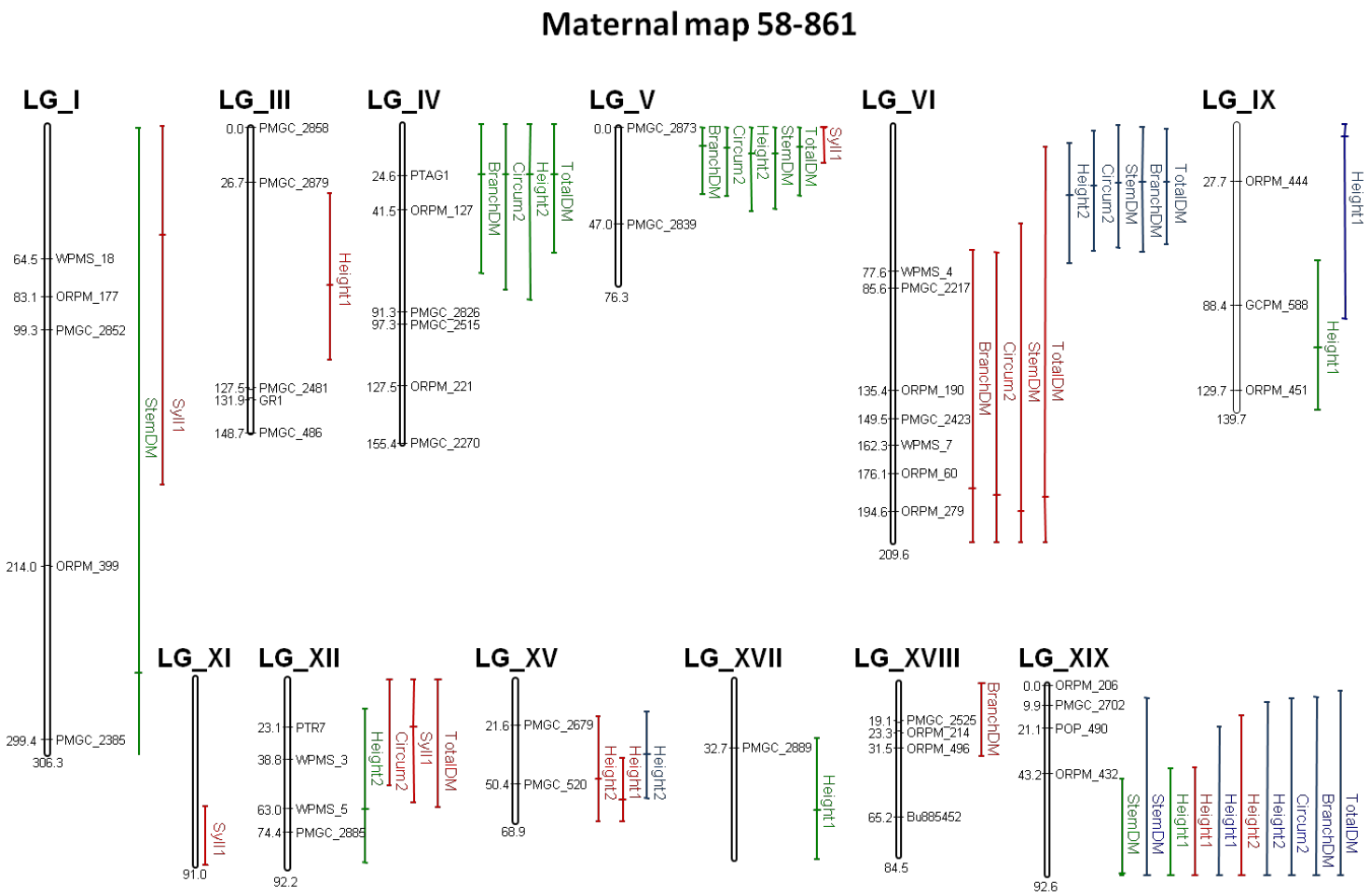

Figure 4.15: QTLs for aboveground biomass traits detected in maternal, “58-861”, map. The length of the LG bars and QTL is proportional to the distance in cM. The middle segment on the line indicates the peak of the QTL, and the total length of the line represents the 95% confidence interval. Names of markers and map distances (cM) are indicated on the right and left, respectively, of the linkage groups. Different colors of QTLs were related to different multi-environment analyses (red for CV 2004 vs. MT 2004, green for SAV 2010 vs. VT 2010 and blue for VT 2010 vs. VT 2012).

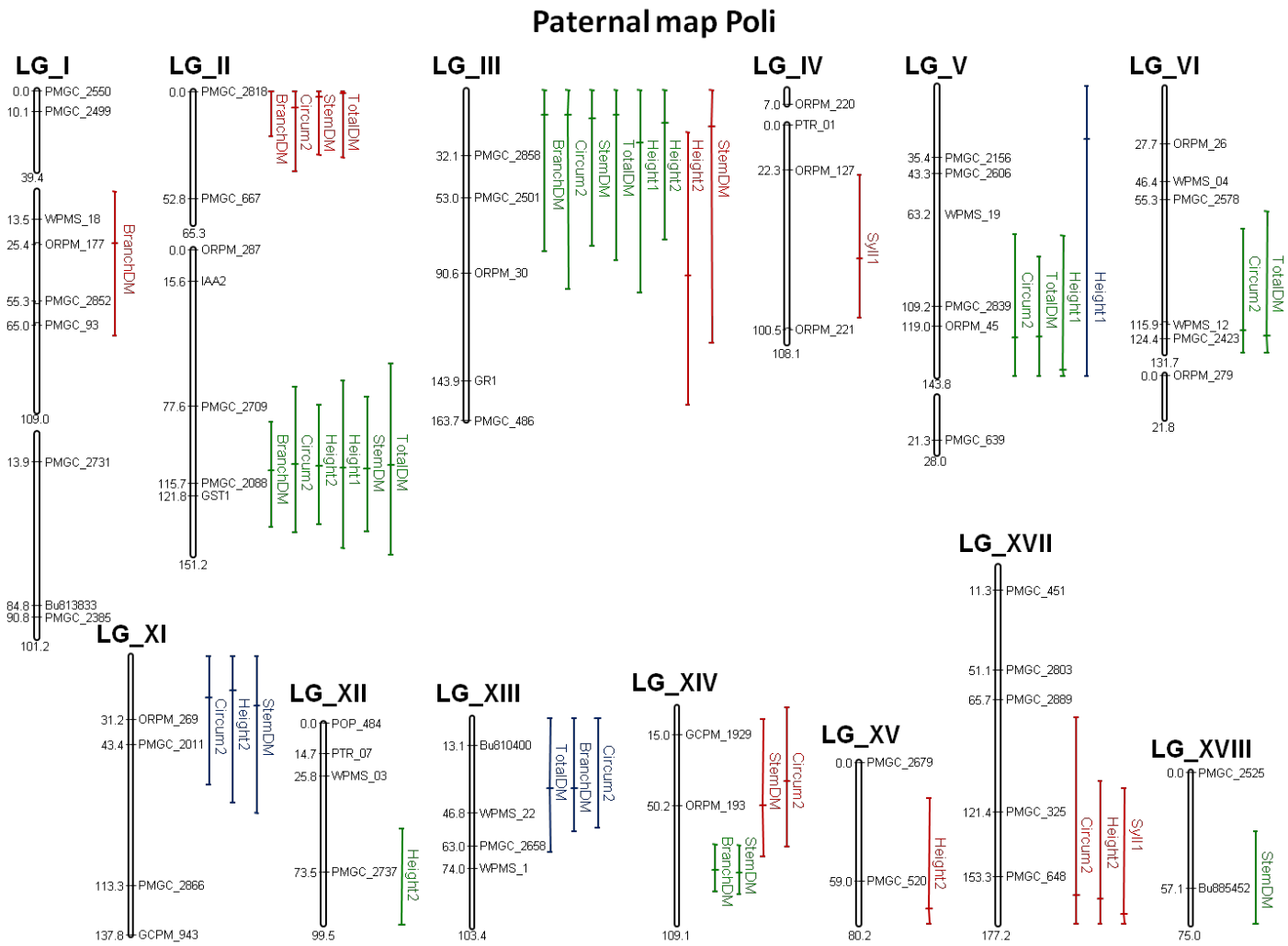

Figure 4.16: QTLs for aboveground biomass traits detected on paternal, “Poli”, map,. The length of the LG bars and QTL is proportional to the distance in cM. The middle segment on the line indicates the peak of the QTL, and the total length of the line represents the 95% confidence interval. Names of markers and map distances (cM) are indicated on the right and left, respectively, of the linkage groups. Different colors of QTLs were related to different multi-environment analyses (red for CV 2004 vs. MT 2004, green for SAV 2010 vs. VT 2010 and blue for VT 2010 vs. VT 2012).

An average of 2.09 and 2.31 QTLs were detected per trait, respectively on the maternal ("58-861") and paternal ("Poli") maps (Tab. 4.14). The individual explained phenotypic variation per traits (PVE_{ind}) ranged between 0.8% and 16.6%. Considering the three multi environment analysis separately (CV 2004 vs. MT 2004, SAV 2010 vs. VT 2010 and VT 2010 vs. VT 2012), the total PVE per trait varied from 0.8% (one QTL for Height2 for VT 2010 vs. VT 2012 on "Poli" map) to 33.1% explained by two QTLs for TotalDM for VT 2010 vs. VT 2012 on "58-861" map (Tab. 4.14).

In order to point out how the detected QTLs are involved in the explanation of the phenotypic variance observed for biomass production (StemDM, BranchDM and TotalDM), the single PVE were clustered in base their values (Fig. 4.17). The great number of QTLs explains less than 10% of phenotypic variance, for both the parents and in more environments. Only 3 QTLs (3.9% of total number of detected QTLs) found on maternal map for VT 2012, explain more than 26% of PVE.

Table 4.14: Number of QTLs per biomass and allometric traits. Total phenotypic explained variation (PVE) by the QTLs in each environment (CV 2004, MT 2004, SAV 2010, VT 2010, VT 2012) is shown. Individual phenotypic explained variation per trait (PVE_{ind}) is the ratio between PVE and number of QTLs.

			CV 2004		MT 2004	
Trait	**Map**	**n. QTLs**	**PVE**	**PVE_{ind}**	**PVE**	**PVE_{ind}**
Height1	Poli	0	-	-	-	-
Syll1	Poli	2	19.4	9.7	4.1	2.1
Height2	Poli	3	16.9	5.6	13.6	4.5
Circum2	Poli	4	29.6	7.4	20.8	5.2
StemDM	Poli	3	20.4	6.8	13.0	4.3
BranchDM	Poli	2	13.5	6.8	13.5	6.8
TotalDM	Poli	2	9.7	4.9	10.2	5.1
Height1	58-861	3	20.3	6.8	12.5	4.2
Syll1	58-861	4	22.3	5.6	28.8	7.2
Height2	58-861	2	10.1	5.1	6.4	3.2
Circum2	58-861	2	10.6	5.3	13.0	6.5
StemDM	58-861	1	3.2	3.2	8.7	8.7
BranchDM	58-861	2	14.4	7.2	11.1	5.6
TotalDM	58-861	2	8.2	4.1	11.0	5.5

			SAV 2010		VT 2010	
Trait	**Map**	**n. QTLs**	**PVE**	**PVE_{ind}**	**PVE**	**PVE_{ind}**
Height1	Poli	2	17.9	9.0	7.9	4.0
Height2	Poli	3	27.9	9.3	11.2	3.7
Circum2	Poli	3	24.8	8.3	9.5	3.2
StemDM	Poli	4	32.1	8.0	14.5	3.6
BranchDM	Poli	3	27.7	9.2	11.0	3.7
TotalDM	Poli	3	23.9	8.0	9.6	3.2
Height1	58-861	3	16.7	5.6	17.0	5.7
Height2	58-861	3	13.9	4.6	11.7	3.9
Circum2	58-861	2	11.8	5.9	10.6	5.3
StemDM	58-861	3	14.0	4.7	13.6	4.5
BranchDM	58-861	2	10.5	5.3	9.6	4.8
TotalDM	58-861	2	11.0	5.5	10.5	5.3

			VT 2010		VT 2012	
Trait	**Map**	**n. QTLs**	**PVE**	**PVE_{ind}**	**PVE**	**PVE_{ind}**
Height1	Poli	1	1.4	1.4	8.5	8.5
Height2	Poli	1	0.8	0.8	12.1	12.1
Circum2	Poli	2	4.6	2.3	18.1	9.1
StemDM	Poli	1	1.4	1.4	7.2	7.2
BranchDM	Poli	1	2.4	2.4	5.8	5.8
TotalDM	Poli	1	2.7	2.7	5.8	5.8
Height1	58-861	2	7.9	4.0	17.3	8.7
Height2	58-861	3	11.7	3.9	32.4	10.8
Circum2	58-861	2	8.8	4.4	32.3	16.2
StemDM	58-861	2	8.1	4.1	32.5	16.3
BranchDM	58-861	2	8.3	4.2	33.0	16.5
TotalDM	58-861	2	8.8	4.4	33.1	16.6

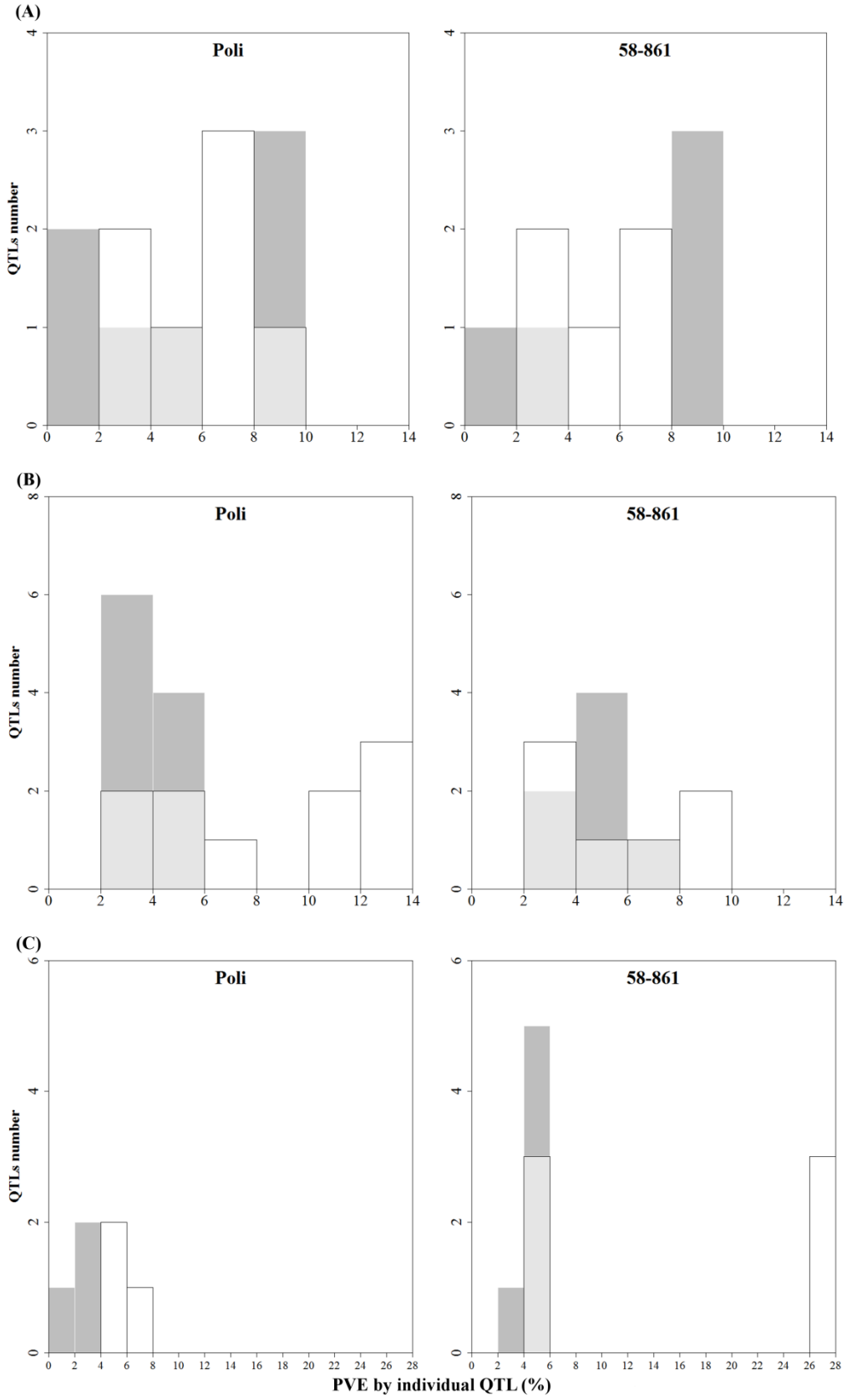

Figure 4.17: Number of detected QTLs and their PVE values for StemDM, BranchDM and TotalDM. The three multi-environment analysis are shown: (A) CV 2004 vs. MT 2004 (grey and white); (B) SAV 2010 vs. VT 2010 (grey and white); (C) VT 2010 vs. VT 2012 (grey and white).

4.5.2. Bud flush phenology

Two multi-environment analysis were performed for the QTL detection: VT 2010 vs. VT 2012 and CV 2004 vs. MT 2004. In the first one analysis (VT 2010 vs. VT 2012), a total number of 39 QTLs were detected for the three onset-of-stage (bf_date1, bf_date1.5 and bf_date2) and the duration of leaf initiation (subproc1), expressed such as temperatures accumulation (CAT_{10} and CMT_5). In the second one analysis (MT 2004 vs. CV 2004), 15 QTLs for the central phase of bud flush (bf_date1.5) were identified. In total, 54 QTLs for both the parental maps were identified, corresponding to 9 genomic regions. Usually, QTL corresponding to the same trait expressed both in CAT_{10} and in CMT_5 overlapped.

On the maternal map "58-861" (Tab. 4.14, Fig. 4.18), 19 and 7 QTLs were found in the two multi-environments analyses (VT 2010 vs. VT 2012 and MT 2004 vs. CV 2004), therefore, a total number of 26 QTLs on nine genomic regions were detected. The phenotypic variance explained by these QTL ranged from 0.1% to 23.8%, with an average of 8.3% across all individual QTL (Tab. 4.14). Total PVE per trait varied from 0% observed for subproc1_CAT_{10} on maternal map, to 34.8% for bf1.5_CMT_5 again to maternal map. The two traits, subproc1_CAT_{10} and bf1_CAT_{10}, presented the lowest and the greatest value of individual PVE (0% and 14.5%, respectively) (Tab. 4.16). Seven QTL had significant differences at VT site between the year 2010 and 2012, and two QTL had significant differences between MT and CV sites in 2004 (Tab. 4.14). A main QTL cluster was found on LG I, composed of 8 QTL representing two genomic regions. Indeed four traits (bf1_CMT_5, bf1.5_CMT_5, bf2_CAT_{10} and bf2_CMT_5) presented two linked QTL on this LG (Tab 4.14, Fig. 4.18). QTL identified on LG I also showed the greatest value of LOD (up to 10.0 for bf2_CMT_5) and PVE.

On the parental map "Poli" (Tab. 4.15, Fig. 4.19) 20 QTLs in VT 2010 vs. VT 2012 and 8 QTLs in CV 2040 vs. MT 2004 were found, corresponding to total 28 QTLs and nine genomic regions. The phenotypic variance explained by these QTL ranged from 0.1% to 14.7%, with an average of 6% across all individual QTL (Tab. 4.15). Total PVE per trait varied from 1.8% observed for subproc1_CMT_5 VT 2010 vs. VT 2012, to 31.8% for bf1.5_$CAT_{10\ in}$ CV 2004 vs. MT 2004. The two traits, bf1.5_CMT_5 and subproc1_CMT_5, presented the lowest and the greatest value of individual PVE (0.8% and 11.6%, respectively) (Tab. 4.16). Eight QTL had significant differences at VT site between the year 2010 and 2012 (Tab. 4.15). Three main QTL clusters were found on LG I, VIII and XIV, composed of 5, 8 and 6 QTL respectively. These QTLs showed the greatest values of PVE (up to 14.2% and 14.7 for onset of bf_date2). Such as for the female parent "58-861", on the LG I two cluster of 2 and five QTL indicated two distinct genomic regions.

Table 4.14: QTL detected in the maternal "58-861" map for traits related to bud flush phenology. For each QTL, the linkage groups (LG), the LOD (logarithm of the odds), the position of peak (in cM), the 95% confidence interval (95% CI) in cM, the percentage of phenotypic variance explained (PVE) at each environment are shown. Two linked QTLs detected for a trait are shown in italic font. LG refers to the map in figure 4.18. Statistical significance (*P-value*) of different weight of the two environments is given (*ns* = non significant, * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$).

CV 2004 vs. MT 2004										
Trait	**LG**	**LOD**	**Peak**	**95% CI**		***P-value***	**CV 2004**		**MT 2004**	
							Effect	**PVE(%)**	**Effect**	**PVE(%)**
bf1.5_CAT_{10}	I	9.2	233.8	213.3	247.3	*ns*	-2.4	18.4	-1.9	5.8
bf1.5_CMT_5	I	5.9	232.9	203.7	254.6	**	-4.1	11.3	-1.1	5.1
bf1.5_CMT_5	II	3.7	151.8	94.5	193.8	*ns*	-0.4	0.1	1.4	8.5
bf1.5_CAT_{10}	XV	5.3	68.9	58.0	68.9	**	-0.6	1.2	-2.8	13.2
bf1.5_CMT_5	XV	6.3	66.2	59.0	68.9	*ns*	-1.9	2.4	-1.8	14.2
bf1.5_CAT_{10}	XVII	3.5	0.0	0.0	67.3	*ns*	-1.4	5.7	-1.7	5.1
bf1.5_CMT_5	XVII	3.1	49.3	0.0	87.2	*ns*	-2.3	3.6	-1.3	7
Mean								6.1		8.4
VT 2010 vs. VT 2012										
Trait	**LG**	**LOD**	**Peak**	**95% CI**		***P-value***	**VT 2010**		**VT 2012**	
							Effect	**PVE(%)**	**Effect**	**PVE(%)**
bf1_CAT_{10}	I	6.1	230.8	144.1	298.4	*ns*	-1.6	9	-2.6	14.5
bf1_CMT_5	I	8.9	19.0 231.0	0.0 139.4	255.2 305.5	*ns* *ns*	0.3 -1.0	9.3	-0.3 -0.4	23.8
bf1.5_CAT_{10}	I	6.1	231.0	127.6	306.2	*ns*	-1.3	9	-3.0	13
bf1.5_CMT_5	I	8.9	18.9 230.9	0.0 133.4	216.8 305.2	*ns* *	0.5 -1.2	9.9	-0.3 -0.4	21
bf2_CAT_{10}	I	8.8	83.1 231.5	0.0 123.5	222.6 306.2	*ns* *ns*	1.0 -1.1	16	0.5 -2.7	12.2
bf2_CMT_5	I	10.0	19.9 230.9	0.0 123.7	223.2 306.2	*ns* *	0.6 -1.2	11.5	-0.2 -0.3	21.7
bf2_CMT_5	II	2.7	76.5	23.8	158.5	*	0.8	4.4	0.1	3.1
subproc1_CAT_{10}	III	4.0	140.0	63.0	148.9	*ns*	-0.9	12.8	0.1	0
subproc1_CMT_5	III	3.9	144.8	108.5	148.9	***	-0.6	11	0.0	1
subproc1_CMT_5	IX	3.1	0.0	0.0	82.5	*ns*	0.4	4.4	0.1	9.3
bf2_CAT_{10}	XIII	2.8	180.8	26.0	180.8	*ns*	0.8	5	1.2	2.3
bf2_CMT_5	XIII	3.2	180.8	38.1	180.8	*	0.9	5.8	0.1	2.5
bf1.5_CAT_{10}	XIX	2.3	19.2	0.0	39.0	*	0.3	0.4	2.6	9.2
bf1.5_CMT_5	XIX	2.4	13.3	0.0	40.9	*ns*	0.5	1.2	0.3	6.8
bf2_CAT_{10}	XIX	3.4	21.1	1.0	33.1	**	0.4	1	2.7	11.8
Mean								7.3		10.1

Table 4.15: QTL detected in the paternal "Poli" map for traits related to bud flush phenology. For each QTL, the linkage groups (LG), the LOD (logarithm of the odds), the position of peak (in cM), the 95% confidence interval (95% CI) in cM, the percentage of phenotypic variance explained (PVE) at each environment are shown. LG refers to the map in figure 4.19. Statistical significance (*P-value*) of different weight of the two environments is given (*ns* = non significant, * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$).

CV 2004 vs. MT 2004										
Trait	**LG**	**LOD**	**Peak**	**95% CI**		***P-value***	**CV 2004**		**MT 2004**	
							Effect	**PVE(%)**	**Effect**	**PVE(%)**
bf1.5_CAT_{10}	Ic	3.1	36.5	0.0	75.3	*ns*	-1.5	7.5	0.1	0
bf1.5_CMT_5	VII	2.5	49.4	7.2	67.4	*ns*	0.7	0.3	-1.2	6.6
bf1.5_CAT_{10}	VIII	4.0	43.1	2.7	87.4	*ns*	1.9	11.9	0.8	1
bf1.5_CMT_5	VIII	4.5	45.1	5.1	81.3	*ns*	3.2	7.1	1.2	6.4
bf1.5_CMT_5	X	2.6	54.9	27.5	82.4	*ns*	0.0	0	-1.5	10.2
bf1.5_CAT_{10}	XII	2.8	53.6	31.4	77.8	*ns*	1.4	6.7	-0.2	0.1
bf1.5_CMT_5	XII	4.1	51.0	37.9	63.8	*ns*	4.4	13	0.0	0
bf1.5_CAT_{10}	XIXb	2.8	1.4	0.0	21.8	*ns*	1.3	5.7	1.1	2
Mean								6.5		3.2
VT 2010 vs. VT 2012										
Trait	**LG**	**LOD**	**Peak**	**95% CI**		***P-value***	**VT 2010**		**VT 2012**	
							Effect	**PVE(%)**	**Effect**	**PVE(%)**
subproc1_CAT_{10}	Ia	2.9	39.4	11.2	39.4	**	1.0	0	-1.7	13
subproc1_CMT_5	Ia	2.2	35.2	0.5	39.4	*ns*	-0.2	1.8	-0.2	11.6
bf1_CMT_5	Ic	3.4	20.6	0.0	63.3	*ns*	-0.6	2.8	-0.3	8.9
bf1.5_CAT_{10}	Ic	2.8	20.6	0.0	56.7	*	-0.6	1.7	-2.4	8.4
bf1.5_CMT_5	Ic	3.4	20.6	0.0	56.6	*ns*	-0.6	2.4	-0.3	9.5
bf2_CMT_5	Ic	2.5	20.6	0.0	59.3	*ns*	-0.5	1.9	-0.2	6.9
bf1_CAT_{10}	VIII	3.7	46.5	22.1	70.1	*ns*	1.5	8.1	1.7	5.8
bf1_CMT_5	VIII	4.1	44.0	13.0	72.9	*ns*	0.9	7.2	0.3	7.9
bf1.5_CAT_{10}	VIII	3.6	46.5	10.9	87.0	*ns*	1.3	8.1	1.7	4.4
bf1.5_CMT_5	VIII	4.0	45.2	15.3	73.8	*	1.1	7.3	0.3	7.2
bf2_CAT_{10}	VIII	2.9	52.6	5.8	99.1	*ns*	0.9	5.3	1.4	3.1
bf2_CMT_5	VIII	3.6	47.9	5.8	92.9	*	1.0	6.3	0.2	5.7
subproc1_CAT_{10}	XII	3.2	19.2	0.0	47.3	*ns*	-0.4	2.6	-1.2	7.4
bf1_CAT_{10}	XIV	3.8	1.0	0.0	36.9	*ns*	-1.5	7.4	-1.8	6.5
bf1_CMT_5	XIV	3.8	0.6	0.0	36.5	*	-1.0	7.6	-0.2	4.5
bf1.5_CAT_{10}	XIV	3.9	3.5	0.0	32.1	*ns*	-1.5	10.8	-1.3	2.7
bf1.5_CMT_5	XIV	4.7	2.3	0.0	29.7	**	-1.4	11.1	-0.2	4.7
bf2_CAT_{10}	XIV	4.6	3.2	0.0	31.8	*ns*	-1.4	14.2	-1.4	3.1
bf2_CMT_5	XIV	5.5	3.5	0.0	23.2	**	-1.5	14.7	-0.2	5.5
subproc1_CAT_{10}	XVIII	2.3	54.7	23.3	74.9	*	1.2	0.2	-1.3	7.8
Mean								6		6.7

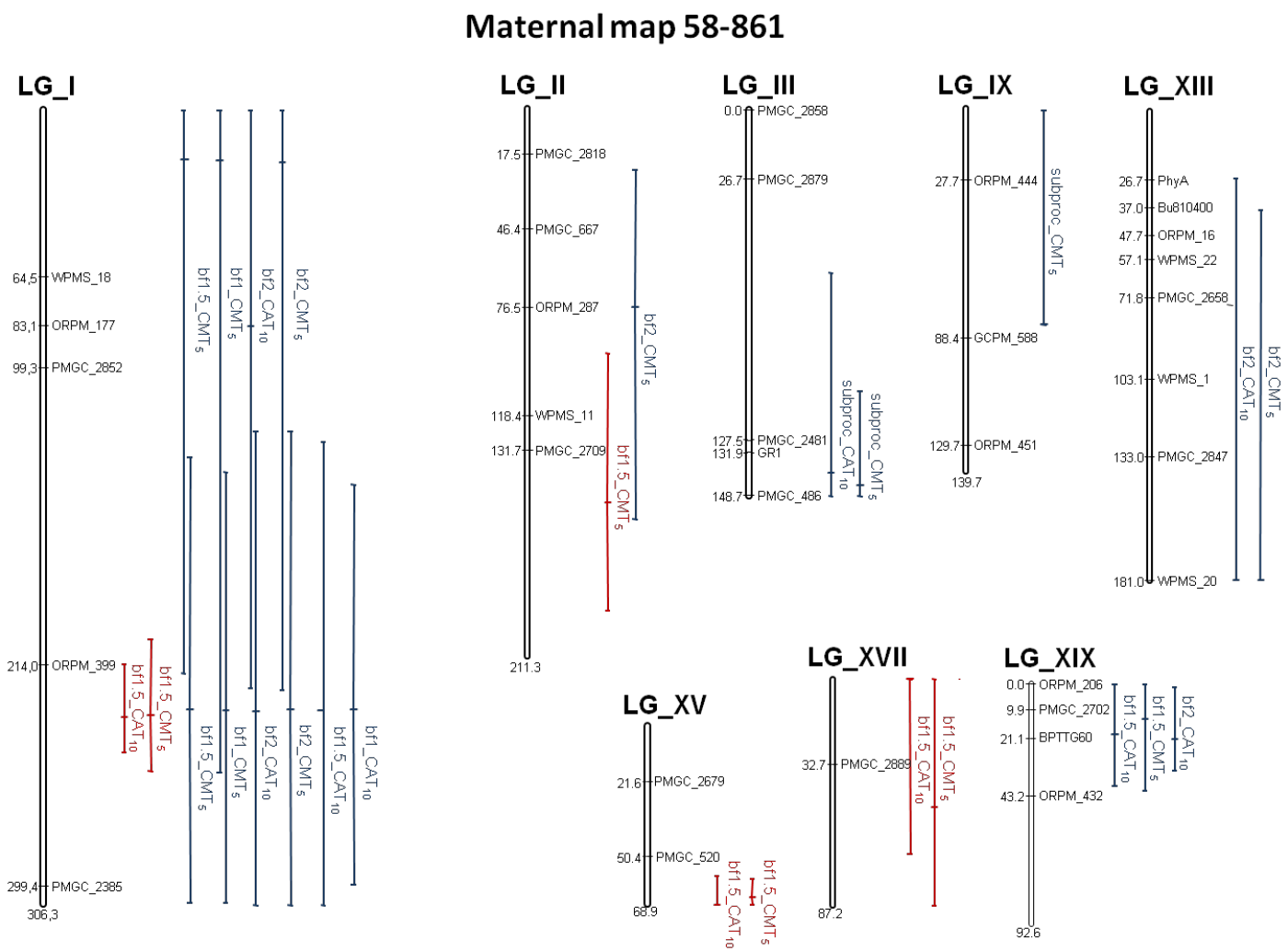

Figure 4.18: QTLs for bud phenology traits detected on maternal map “58-861”. The length of the LG bars and QTL is proportional to the distance in cM. The middle segment on the line indicates the peak of the QTL, and the total length of the line represents the 95% confidence interval. Names of markers and map distances (cM) are indicated on the right and left, respectively, of the linkage groups. Different colors of QTLs were related to different multi-environment analyses (red for CV 2004 vs. MT 2004 and blue for VT 2010 vs. VT 2012).

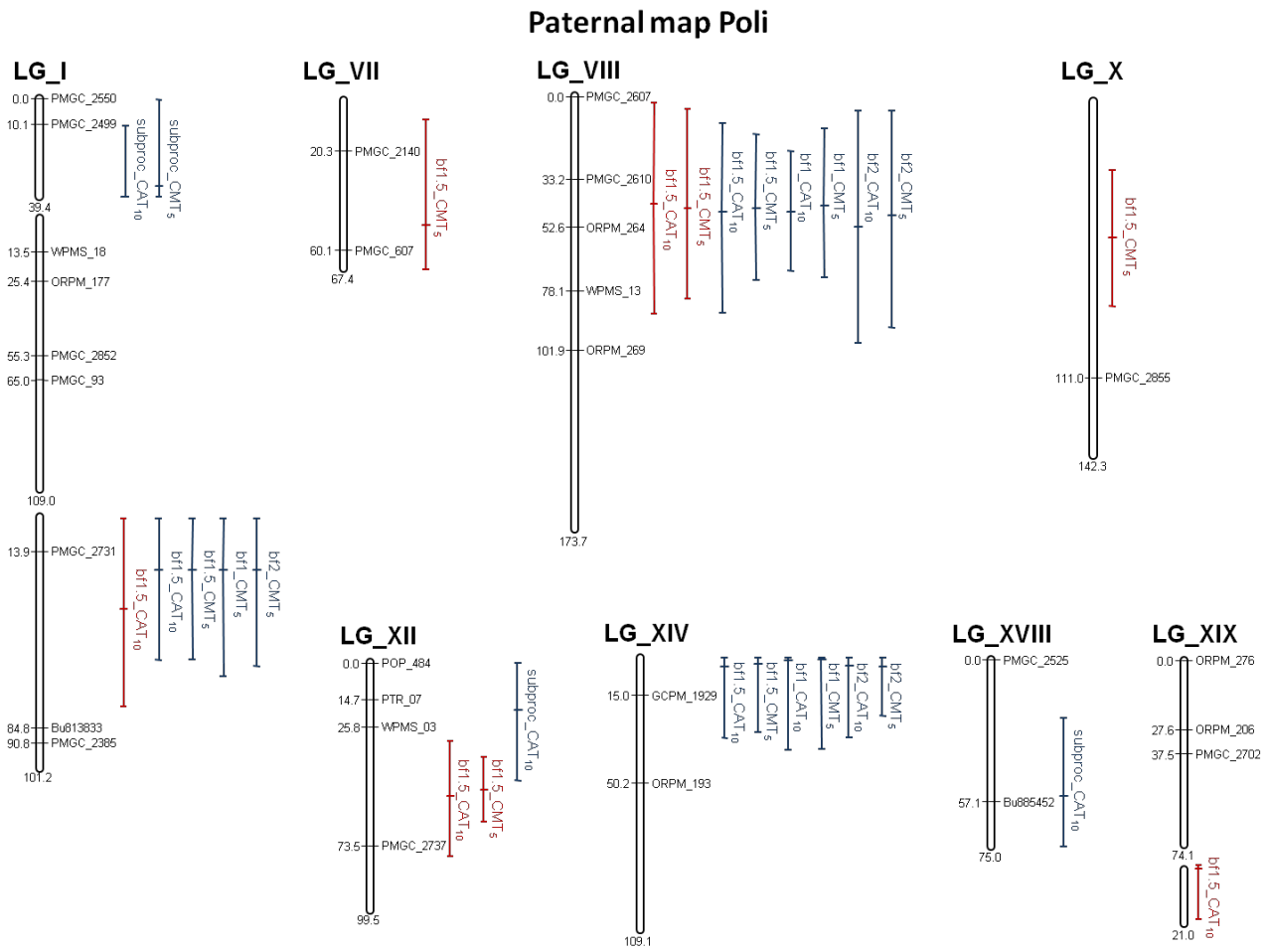

Figure 4.19: QTLs for bud phenology traits detected on paternal map “Poli”. The length of the LG bars and QTL is proportional to the distance in cM. The middle segment on the line indicates the peak of the QTL, and the total length of the line represents the 95% confidence interval. Names of markers and map distances (cM) are indicated on the right and left, respectively, of the linkage groups. Different colors of QTLs were related to different multi-environment analyses (red for CV 2004 vs. MT 2004 and blue for VT 2010 vs. VT 2012).

Table 4.16: Number of QTLs per bud phenology traits. Total phenotypic explained variation (PVE) by the QTLs in each environment (CV 2004, MT 2004, VT 2010 and VT 2012) is shown. Individual phenotypic explained variation per trait (PVE_{ind}) is the ratio between PVE and number of QTLs.

			CV 2004		**MT 2004**	
Trait	**Map**	**n. QTLs**	**PVE**	**PVE_{ind}**	**PVE**	**PVE_{ind}**
bf1.5_CAT10	Poli	4	31.8	8.0	3.1	0.8
bf1.5_CMT5	Poli	4	20.4	5.1	23.2	5.8
bf1.5_CAT10	58-861	3	25.3	8.4	24.1	8.0
bf1.5_CMT5	58-861	4	17.4	4.4	34.8	8.7

			VT 2010		**VT 2012**	
Trait	**Map**	**n. QTLs**	**PVE**	**PVE_{ind}**	**PVE**	**PVE_{ind}**
bf1_CAT10	Poli	2	15.5	7.8	12.3	6.2
bf1.5_CAT10	Poli	3	20.6	6.9	15.5	5.2
bf2_CAT10	Poli	2	19.5	9.8	6.2	3.1
subproc1_CAT10	Poli	3	2.8	0.9	28.2	9.4
bf1_CMT5	Poli	3	17.6	5.9	21.3	7.1
bf1.5_CMT5	Poli	3	20.8	6.9	21.4	7.1
bf2_CMT5	Poli	3	22.9	7.6	18.1	6.0
subproc1_CMT5	Poli	1	1.8	1.8	11.6	11.6
bf1_CAT10	58-861	1	9.0	9.0	14.5	14.5
bf1.5_CAT10	58-861	2	9.4	4.7	22.2	11.1
bf2_CAT10	58-861	4	22.0	5.5	26.3	6.6
subproc1_CAT10	58-861	1	12.8	12.8	0.0	0.0
bf1_CMT5	58-861	2	9.3	4.7	23.8	11.9
bf1.5_CMT5	58-861	3	11.1	3.7	27.8	9.3
bf2_CMT5	58-861	4	21.7	5.4	27.3	6.8
subproc1_CMT5	58-861	2	15.4	7.7	10.3	5.2

The PVE distribution in different classes of width showed a similar pattern observed for traits related to biomass production, with a greater number of QTLs to explain lower values than higher values of phenotypic variation (Fig. 4.20). For both the environments (VT 2010 vs. VT 2012) and both the climatic parameters (CAT_{10} and CMT_5), parent "Poli" showed more QTLs with lower values of PVE. Differently, "58-861" showed more QTLs with PVE values greater than 10%, with maximum value of 23.8% observed for VT 2012.

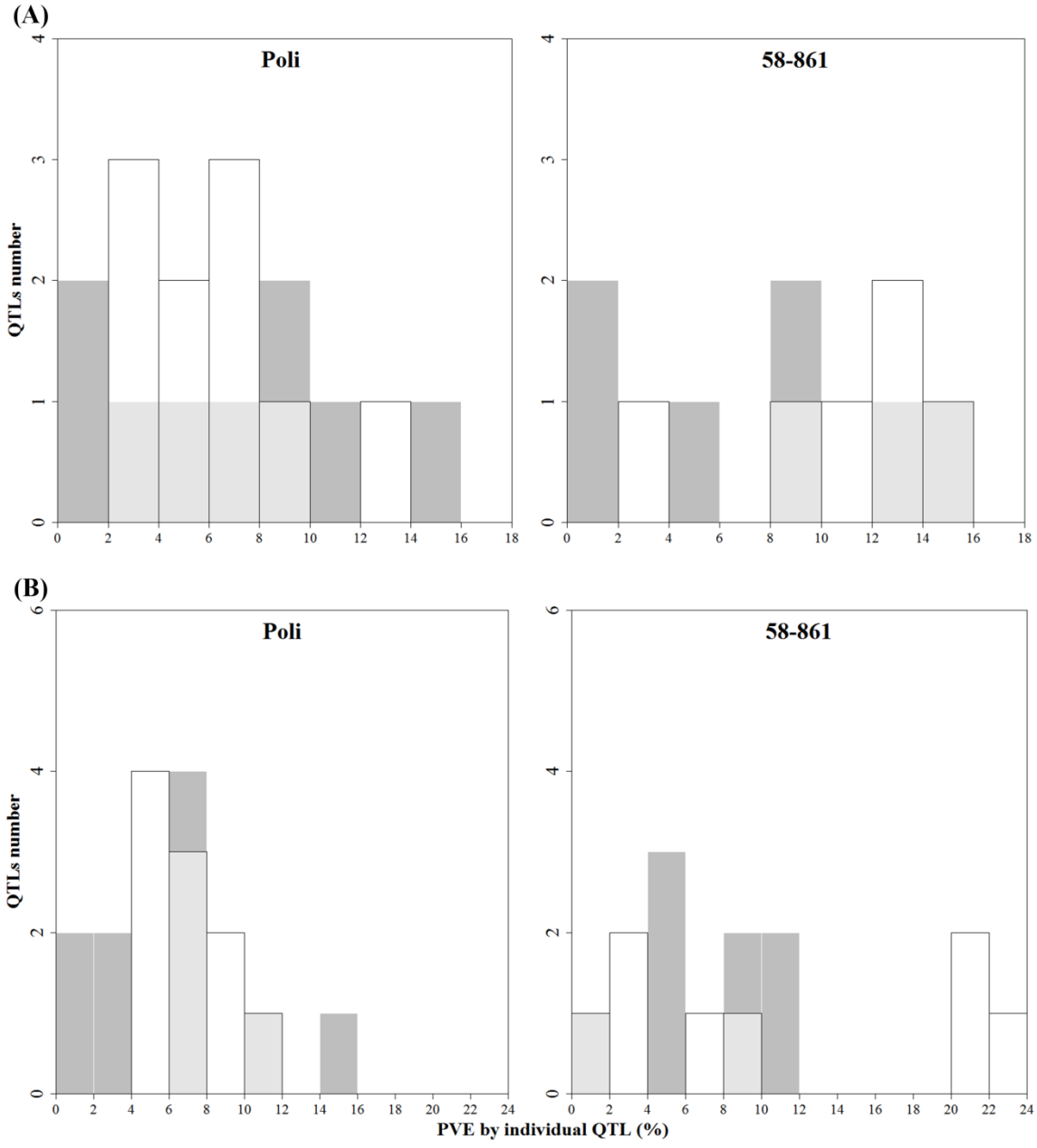

Figure 4.20: Number of detected QTLs and their PVE values for bud flush selected traits. The results of the multi-environment analysis between VT 2010 and VT 2012 are shown. Onset-of-stage and subprocess duration traits expressed by CAT10 (A) and CMT5 (B) are shown separately. Grey bars represent PVE values related to VT 2010 and white bars represent VT 2012.

5. DISCUSSION

5.1. Aboveground biomass production

The present study explored the phenotypic variation and the genetic architecture of biomass production and allocation in a *P. nigra* pedigree (POP5) of 162 genotypes, obtained from two parents characterized by different ecological and morphological traits and provenances by divergent climatic conditions. Multiple linear regression analyses were performed to estimate biomass production in different environmental conditions.

The equations established for each environment were adequate to catch the genetic variation available within the POP5 family as the obtained biomass estimations were used in descriptive, inferential and QTL analysis. Linear regression models and analyses of covariance assume normal distribution of the dependent variable and homoscedasticity of its variance, so different transformations were needed. Circumference and height significantly contributed to the equations in a most of the cases, however the contribution of the latter trait was generally poor and there were only slight improvements concerning the coefficient of determination (R^2) when this predictor was added in the model. Number of sylleptic branches is closely related to tree biomass (Scarascia Mugnozza et al., 1997; Rae et al., 2004); however, as a predictor, it was significant only in one case. So, even if the contribution of sylleptic branches in the prediction equations is often of minimum impact, it can be considered an interesting selection criterion in breeding programs for poplar (Marron et al., 2006; Rae et al., 2007). This is in line with previous studies where stem circumference (or diameter) was a reliable and sufficient predictor for estimating dry weight of tree (Ketterings et al., 2001; Ritson and Sochacki, 2003, Dillen et al., 2009). Consequently, model based on circumference (or diameter) alone must be preferred because sampling of this trait is less complicated, and time- and material-demanding than the measurement of stem height or number of branches (Wang and Kimmins, 2002). With the exception of the analyses performed between years (VT 2010 vs. VT 2012), the ANCOVA showed significant environmental effects on the biomass estimation. Moreover, in some cases it was significant the interaction between covariates (circumference or height) with the environment, so it was not possible to simplify the equation model among sites. Differently, in VT 2010 vs. VT 2012 it was possible to unify biomass estimation models between the years thanks to similar biomass values. The traditional allometric model ($Y=aX^b$) widely used in biomass studies, may be precise enough for tree biomass, but is often unsatisfactory for canopy biomass components such as branches. The equations developed in the present work showed a high level of accuracy with only a 2% of mean percentage bias, similar

to the bias level (2.22%) observed by Dillen and coworkers (2007). Tuskan and Rensema (1992) highlighted that a small degree of discrepancy between observed and predicted biomass values can lead to a high percent of bias. Luckily, this bias did not occurred in this work thanks to high precision and carefulness used during the sampling activity in the fields.

A large phenotypic range was observed in terms of biomass production, both within and among environments. The POP5 family was very productive in terms of tree dry weight. Under favorable conditions it produced between 5000 and 6000 g of aboveground individual tree dry weight in the central Italian site after two growing seasons. In this site, a medium rate of survival (around 80%) was observed, with an interesting yield production of 11.3 t ha^{-1} $year^{-1}$. Considering the high survival rates of stools (>80%) and the obtained constant dendromass yield, never lower than 10 t ha^{-1} $year^{-1}$ over frequent and repeated coppicing, the performances showed by POP5 (intra-specific controlled cross of *P. nigra*) could be considered not negligible. Moreover, the scientific rather than the economic objectives of the trials and the following limitation of irrigation and fertilization, caused probably a lower production of biomass. Low values of AWP_{real} have been reported for various 2-year-old Euramerican and Interamerican hybrid poplars: 5.5 t ha^{-1} $year^{-1}$ (Laureysens et al. 2003), 4.5 t ha^{-1} $year^{-1}$ (Laureysens et al. 2004), 1.3 t ha^{-1} $year^{-1}$ (Wullschleger et al., 2005), 5.7 t ha^{-1} $year^{-1}$ (Marron et al., 2010a), 4.1 t ha^{-1} $year^{-1}$ (Marron et al., 2010b). Hence the growth potential of the POP5 family was very interesting for the use of germplasm resources of black poplar in genetic improvement programs. Perhaps no other tree genus in temperate climates can match the biomass accumulation potential of inter-american hybrids grown under favorable conditions (Eckenwalder 2001), but in front of the result of this study it is possible to extent this sentence also to our family of *Populus nigra* for Mediterranean environments.

As might be expected, different age root system widely affected the tree total dimension and field production observed in the trials. Nowadays, coppicing as mean to increase biomass production has been well studied in poplar plantations. In a study on a hybrid poplar growing under a 3-year cutting cycle, Bedeneau and Auclair (1989) found that in the first year after coppicing, the aboveground biomass increment was quite low while root growth was increased. In conformity with the observed values reported in bibliography, the lack of coppicing at the end of the first growing season in CV and MT sites, influenced negatively the yield performances observed at the end of second biennial rotation. Differently, coppicing at the end of the first growing season of the biennial rotation caused an “additive” effect on the root system development and increasing the aboveground biomass production , as observed in SAV and VT sites. Moreover, for CV and MT sites, the severe drought condition occurred in the year of plantation (i.e. 2003) influenced the growth observed at the end of

second growing season. At the same time, the higher yield observed in SAV and VT, as might be expected, was due to the greater soil fertility of the northern site and to the better rainfall annual regime occurred in the period 2009 - 2012 in VT. Concerning the results observed in VT, trial of 2012 (5 years-old root system) shown greater individual tree dimension and lower aboveground wood production realized than trial of 2010 (3 years-old root system). These results can be partially explained by the rising of mortality rate occurred between the years 2010 and 2012 (around 22%), and the subsequent increment of spacing among trees. It was generally accepted that wider spacing produces larger trees, but productivity per hectare can be reduced (Proe et al., 1999). Moreover, Armstrong et al. (1999) observed that a maximum annual volume increment peaked at 5 years and that the production declined sharply beyond this point. In front of the bibliography and the evidence pointed out by this work, the choice of the best rotation length is very important for to maximize the biomass production.

About the two parents, “Poli” generally showed lower values of tree dimensions (diameter and height) than other genotypes in the different environments. Taking into account the great differences observed among experimental conditions (i.e. roots age, annual temperature trends and rainfall regime), it is possible to state that “Poli” has low adaptability to divergent environments. In the northern Italian sites, quite far from the origin site of Policoro, “Poli” was not well adapted to the climate condition, in fact it did not survive after coppicing (50% of survival rate in SAV) as the northern parental genotype. This limited capacity of adaptation to diverse pedo-climatic conditions was also observed in a previous work of Fabbrini and coworkers (2012). Actually, after a coppicing occurred in 2005 in CAV, all the replicates of “Poli” did not re-sprout in the following spring. Moreover, “Poli” shown lower values of weight, circumference and height than mean family values in the southern sites of MT and VT and this result suggested how “Poli” was strongly penalized by the lateral competition of the nearest clones. However, results obtained in MT 2004, with “Poli” that showed a performance comparable to the family mean and to “58-861” production, provided information about growth occurred in response to drought stress in 2003. In fact, the comparison between the ‘drought-tolerant’ “Poli” and the ‘drought-sensitive’ “58-861” clones revealed that the latter clone used water with greater efficiency but required more water than the male parent also during period of water scarcity. On the other hand, in an environment with severe drought “Poli” responded promptly to stress conditions by activating drought-tolerance mechanisms, whereas “58-861” was unable to avoid water stress damage in time (Cocozza et al., 2010). In this contest, it was clear as “58-861” had greater advantages than “Poli” in temperate, fertile and wet environments, whereas “Poli” shown greater adaptability than “58-861” in warmer and dry locations.

A very large proportion of the high woody biomass production of the F_1 family was due to the branch component, highlighting the potential interest of this family for the production of biomass for fuel rather than for the production of veneer or saw wood. The high branch production of this family was probably due to the origin of the parents. The male parent "Poli" has its origin in southern Italy and is characterized by a larger branch production as compared to parent of northern provenance. In POP5, a third of total biomass was invested in branches (at mean 35% of the total biomass) and higher percentage of wood accumulated in branches exceeded 44% at the MT site. Comparison among 1-year-old and 2-year-old genotypes reveal marked age-related changes in patterns of biomass distribution (Wullschleger et al., 2005). Moreover, King and coworkers (1999) point out that as plants increase in size and age, the relative distribution of biomass among organs frequently changes. The results observed in this work were in agreement with the conclusions above, taking into account that the root system with different age (i.e. 2, 3 and 5 year-old) can explain the lack of a pattern for biomass allocation in the different environments. However, there were previous reports for 2-year-old hybrid or pure species of poplars, indicating that total biomass is distributed among plant components in a manner similar to that observed in this study: 63% stem – 37% branches (Wullschleger et al., 2005), 75% stem – 25% branches (Marron et al., 2010a), 63% stem – 37% branches (Dillen et al., 2007).

Compared to other biomass traits studied in this work, greater values of individual heritability were observed for the number of sylleptic branches in CV and MT (0.27 and 0.36). The same pattern was observed in other papers (Bradshaw and Stettler, 1995; Rae et al., 2004; Rae et al., 2008), with H_i^2 values of the number of sylleptic branches greater than circumference, height or tree weight. These results remarked the strong genetic control on the number of sylleptic branches produced by a tree, and as this trait could be suitable for discriminating the most promising genotypes in term of vigour. However, sylleptic branches production is known as a very plastic traits and the interaction between genotype and environment has been reported to be important for branch and canopy traits (Rae et al., 2008). In this study, significant genotype by environment interaction was observed for the number of sylleptic branches and for their dry weight. Hence, if production of sylleptic branches in many species has been shown to be highly variable under differing environmental conditions (Costes and Guedon 2002), the wood allocation between the main stem and the tree crown has been shown to be stable in different environments. Very low broad sense heritability was observed for other biomass traits such as circumference, height, and aboveground biomass, with lower values than those observed in other studies. Bradshaw and Stettler (1994) observed values greater than 0.60 for height and basal area while Dillen and coworkers (2009) observed individual heritability greater than 0.40 for height and circumference. H^2 values greater than 0.40 were reported for total

aboveground biomass in Marron et al. (2010b) and greater than 0.60 for total dry biomass in Rae et al. (2008). Heritability values cannot be extrapolated to different environments or species as they reflect the expression of genetic variability in a given environment for a specific population (Lynch and Walsh 1998). However, heritability values of comparable traits are often remarkably similar in different populations and even in different species (Visscher et al. 2008).

In the present work, no general pattern of relative importance by source of variance was observed among environments, genetic basis and their interaction. However, the greatest proportion of variance accounted by environmental and residual (i.e. unexplained) sources remarks that; biomass production is a result of biotic and abiotic factors which cooperate during a long time period, so the genetic effect of the plant material is easily hidden. In CV 2004 vs. MT 2004 (i.e. different location and same year, without coppicing) significant G×E interaction was observed for all the studied traits, while in SAV 2010 vs. VT 2010 trial (i.e. different location, the same year, with coppicing) and VT 2010 vs. VT 2012 (i.e. the same location, different growing seasons) no significant interaction was observed for most of the traits. These results can be explained by a different site quality (greater in CV than MT) and rainfall regime (marked dry season in MT than in CV), whereas SAV 2010 vs. VT 2010 trials were characterized by a greater uniformity in climatic and local conditions. What we have seen in SAV 2010 vs. VT 2010 was amplified in VT 2010 vs. VT 2012, where the site quality was almost the same and the climatic conditions not change on the long period. Moreover, the role of coppicing in the determination of the genotypes production in different sites cannot be excluded. When G×E interaction was significant, the extent of the effect was very low (average mean values of 5.44%), with overall values of Spearman's coefficient of 0.33 and a *P*-values always significant (*P*-value≥0.05). These results suggest that genotypes with a superior performance in a site were also highly performing in the other one, hence the interaction was due more to a scale than a ranking effect (i.e. different performances in the sites, without changes in relative position among genotypes). Only 16-17 (13.4%) genotypes of the POP5 family explained at least 50% of the total relative ecovalence, showing that a very low number of genotypes explained the observed interaction effects. Hence, the POP5 family showed to be a productive cross with stable genotypes in the studied environments. This result represents a practical tool applicable in tree breeding strategies and in the genetic improvement providing the possibility to select stable productive genotypes to be utilized in different environments avoiding the risk of unexpected performances.

Many important economic and adaptive traits in forest trees appear to be under the control of multiple genes making conventional breeding very difficult. It is very important to characterize the

growth process at molecular level especially in a breeding program context in order to find the right compromise between the maximization of the growth and the necessity to assure stable performances in different environments. The variation of quantitative growth traits can be dissected in biomass compartment (stem, branches, total volume etc.) and genomic regions involved in the specific expression of the trait of interest can be mapped by quantitative trait loci (QTLs). Furthermore, the dissection of G×E interaction into its individual genetic components, allow to explore the genetic complexity of the phenotypic responses to the environments and should be understood in terms of underlying QTL and their allelic composition (El-Soda et al., 2014). In other words, G×E will correspond to QTL with environment-specific effect or QTL by environment interaction (Q×E) (Boer et al., 2007; Bernardo 2008; Malosetti et al., 2013). In order to achieve this objective, more analyses among different environmental conditions (years, sites and silvicultural practices) were performed in the present study to find clear patterns in QTLs localization on the 19 chromosomes of *Populus* genome. In this work, the number of the mapped QTLs varied among LGs. As expected, correlated traits (i.e. Pearson phenotypic correlation values greater than 0.85) showed QTLs to collocate on the same genomic regions. Even if phenotypic correlations did not provide robust results such as genetic correlations, it can be assumed that the collocation of QTL for traits that showed high genetic or phenotypic correlations showed pleiotropic effects (Rae et al., 2008). In this study height, circumference and biomass were seen to have high phenotypic correlation and collocation of QTL, showing these traits to be useful for selection towards an overall stem growth.

The linkage groups II, III, V, VI, and XIX showed the greatest density of detected QTLs (at least 9 QTLs). The common marker on LG IV, LG V, LG VI and on LG XV between maternal and paternal maps indicates that the identified QTL on these LGs for the two parents are in the same genomic regions. Moreover, QTL obtained by the three multi-environments analyses co-localized on LG VI for circumference and total dry weight and on LG XIX for height of the first year. The LGs with QTL identified in this study were also pinpointed in other published papers. Indeed, Rae and coworkers (2008) found QTLs for the same traits, with a similar high density on LG V (nine QTLs) and LG XII (five QTLs). Muchero and coworkers (2013) identified on LG VI suggestive QTLs associated with height and diameter. Monclus and co-workers (2010) found seven QTLs related to circumference, height and number of sylleptic branches on LG V. Rae and co-workers in 2009, mapped consistent QTLs across multiple coppice cycles and identified five robust QTL hotspots on LG III, IV, X, XIV, and XIX, calling these “Poplar Biomass Loci”. In this study, LG III for the paternal map and LG IV and LG XIX for the maternal map also present a cluster of QTL, eight QTL co-localyze on LG III, four on LG IV and ten on LG XIX. While multiple co-locating

QTLs explaining an high percentage of phenotypic variance for height and diameter, were identified on LG X by Rae and co-workers (2009) , no QTL were identified on this LG in our study.

The common SSR markers among the two maps of our study and the two maps of the Monclus and co-workers (2010) allow identifying QTL localized on the same genomic regions. Indeed, there were six common SSR markers, PTAG1 and ORPM_127 on LG IV, PMGC_2839 on LG V, PMGC_2423 and ORPM_190 on LG VI and PMGC_2679 on LG XV within the QTL interval identified in the two studies, which delimited common genomic regions for height, circumference and number of sylleptic branches.

The large number of QTLs detected, characterized by small or modest effect (overall mean of PVE around 6%) highlights the conventional “polygenic model” for biomass traits. However, a not negligible number of QTLs with PVE greater than 10%, and a low number of QTLs detected for each trait suggest the possibility of “oligogenic model” control (i.e. a few number of QTLs with moderate values of PVE) (Bradshaw and Stettler 1994). The mean values of PVE observed for the different traits studied here suggest that likely many QTLs with small effect or localized in genomic regions which were not covered by the genetic maps, remain undetected.

Total mass of a tree is the cumulative result of carbon assimilation and allocation over multiple seasons of growth. Since both assimilation and allocation are influenced by many biochemical pathways, physiological processes and anatomical traits, as well as environmental variables such as water and nutrient status, light intensity and duration, air and soil temperature, day length and heat sum and perhaps pest and pathogen pressure, it is intuitive that the integration of growth with these variables must be genetically complex (Bradshaw and Stettler, 1994).

5.2. Bud flush phenology

The present study also explored the phenotypic variation and the genetic architecture of bud flush phenology in a pedigree of 162 genotypes, obtained from two parents characterized by different ecological and morphological attributes and provenances by divergent climatic conditions. For this purpose a specific protocol defined to dissect the process into different stages involved in the process and to evaluate the duration of leaf development as separate characters was used to understand the relative contribution of each developing step through an in-depth analysis.

The original protocol by Castellani and coworkers (1967), developed on hybrid poplars in order to link timing of bud flush steps with timing of pest attacks, was adapted to study phenotypic variance shown by POP5 family in response to different climatic inputs. High resolution pictures of each

stage, the definition of the stage duration with the identification of a central subprocess and the rename of the stage in agreement to the protocol proposed for bud set by Rhode et al. (2011), contribute to provide a useful tool to characterize the apical meristem development during the annual life cycle of a tree. As of today, the monitoring of the whole bud flush process repeated in more growing seasons, is an interesting case of study that provides information on the temperature effect on the whole process dynamics. In fact, in many published papers only one phase was taken into account (Tsarouhas et al., 2003; Marron et al., 2010b; Soolanayakanahally et al., 2013; Olson et al., 2013; Ghelardini et al., 2014) and often the whole process was "trivialized" in only this single stage. The utilization of different thermal parameters (i.e. cumulative daily temperatures) provided results related to temperature trend and effects during the bud flush process. Often the first day of thermal accumulation was chosen arbitrarily, set to January, 1st because it is the first day of the year. On the basis of the results of Fabbrini et al. 2012, it was considered that the previous development stage (apical bud fully closed) was completed within November, 1st, and this date was chosen as the first day of thermal accumulation for further study on the temperature influence on bud flush. This approach of analyses allowed temporal continuity between autumnal and springtime bud phenology, taking in consideration the effects of low temperatures occurred between the arrest of the growth and the following spring flushing.

The different trends of temperature occurred in 2010 and 2012 in VT site created two particular cases of comparison: the year 2012 with stable climatic conditions, such as a not perturbed "control plot", with average temperatures never lower than 10 °C during the bud flush process, and the year 2010 with a decreasing temperature trend and an average temperature lower than 10°C for many days during the phenomenon. In response to these two environmental states, POP5 family showed larger phenotypic variance for the different onset-of-stages and stage durations for VT 2010 rather than for VT 2012. Moreover, the beginning of the growing season (GS) was determined like a period of at least 15 days with mean temperature equal or higher than a selected threshold (Perini et al. 2004), here hypothesized of 10 °C. GS was identified in March, 20th in 2010 and in March, 12th in 2012, that are 3 and 4 days, respectively, before the first manifestation of the bud flush process. Taking in consideration the results related to bud flush beginning (bf_date0.5), very similar values of critical temperature (CAT_{10}) were observed in the year 2010 (77.1 °C) and year 2012 (81.8 °C), while around 100 °C of variance were observed between the two studied years for cold accumulation (CMT_5). In synthesis, bud flush timing was largely a function of temperature in most temperate and boreal trees, such as poplar, and buds can subsequently flush in response to a particular heat sum (Soolanayakanahally et al., 2013) accumulated above a certain threshold (Howe et al., 2003) identified in 10°C for POP5 family.

Greater phenotypic variance was observed in 2010 than in 2012, the former year characterized by a drastic fall of minimum temperature below 5°C (i.e. increment of CMT_5 parameter) during the central stages of bud flush. As a consequence, greater variances were observed for bf_date1.5 and bf_date2, with 13 and 14 days of variance between the earliest and the latest genotypes. In 2012 during the first stages of process (bf_date0.5 and bf_date1), the minimum temperature slightly decreased below 5°C, but only for few days. Hence, in 2012 POP5 family showed lower maximum variance (10 days of difference), which decreased with the progression of the stages accordingly to the stabilization of the minimum and the average temperature above 5°C and 10°C, respectively. This could be explained by the different degree of development of the tissues exposed to cold temperature. In fact, in the first part of the bud phenology the scales were closed or slightly opened, so the preformed leaves were protected by the thermal stress. Otherwise, during the second part of the development process, the juvenile leaves were totally exposed to the cold air, that had a greater influence on the process dynamics. This evidence was conformed to the general theory that cell division and growth of the bud are temperature dependent, so that the rate of development towards bud burst and growth onset increase with the rising of air temperatures (Hänninen and Tanino, 2011) and decrease with the reduction of temperatures. Despite the environmental conditions of each trial and taking into account observed phenotypic correlations, all the different phases were strongly correlated each other indicating that once the process starts, all the different following phases, describing a developmental sequence, proceed in a "cascade" fashion. This behavior is probably due to the control of the different traits by the same genes.

Southern parent "Poli" showed great vulnerability to the changes of temperatures, pointing out to considerable transgressive segregation (i.e. without showing greater or lower performances compared to F_1 offspring in 2010. This characteristic was absent in 2012 where "Poli" was among the earliest genotypes of the different stages for the largest part of the process (until to bf_date2). Moreover, "Poli" started the process before "58-861" in all the trials, with only 0.97°C (VT 2010) and 2.59°C (VT 2012) of CAT_{10}, respectively, to achieve the onset of bf_date0.5 after the beginning of growing season (GS). In 2010, "Poli" showed the greatest genotypic mean for duration of bf_date1.5 and bf_date2 (i.e. duration of the leaf initiation), in response to low temperatures occurred around bf_date1.5, with the final result to achieve the onset of bf_date2.5 (i.e. end of bud flush) after the parent "58-861". Otherwise, the northern parent "58-861" showed a broad "indifference" (i.e. very low vulnerability) to changes in temperature. In both years, "58-861" were among the genotypes that required more days to complete the bud flush process. Moreover, after the attainment of the threshold air temperature, the maternal parent required greater CAT_{10} (13.86°C in VT 2010 and 17.67°C in VT 2012) than "Poli" to start the bud flush. At the same time, "58-861"

showed similar time duration of stages, due to the requirement of pronounced changes in temperature to rule the speed of process. These results could be explained taking in consideration the parental site of origin. Genotypes such as “58-861”, from colder climates (e.g. high elevation, northern latitude and continental climate), when reach the critical threshold air temperature to break the bud have the ability to flush even if temperatures are still cool, and can flush earlier than southern genotypes (Howe et al., 2000). This behavior is an adaptation to colder climates where a short growing season limit the growth and the development, especially if late spring frosts are relatively uncommon (Farmer and Reinholt 1986). At first glance, the results presented in this study are in contradiction with the bibliography. It was observed that northern genotypes of boreal species (i.e. western hemlock, amabilis and subalpine fir, and american aspen) grown in southern test plantation with mild winters (i.e. “58-861” grown in VT site) flushed later (Kuser and Ching 1980; Worral 1983; Brissette and Barnes 1984). Otherwise the common later flushing habit of the southern and maritime genotypes, such as “Poli”, seems to be an adaptation to climates with mild winter and long spring with a warm spell, which would promote the bud break, followed by a damaging frost (Campbell and Sugano 1979). It is possible to hypothesize an environmental effect due to the shifting of parents from native areas (southern latitudes) to the site of experimentation (northern latitudes) to explain the observed early bud flush of “Poli”.

The timing of bud flush is primarily controlled by temperature (Juntilla 1989), although secondary environmental factors such as photoperiod and winter chilling requirement can influence the process (Howe et al., 2000). Vitasse and Basler (2013) proposed a theory for the role of photoperiod, chilling requirement and forcing temperature (i.e. heat sum) to explain spatial and temporal variations in bud burst dates, summarized in two main different ways in which photoperiod may interact with temperature:

1) a fixed photoperiod threshold might be required to trigger dormancy release, subsequently allowing buds to respond to forcing temperature with a forcing requirement depending on the chilling fulfillment;

2) the forcing requirement for bud burst might decrease towards its minimal value when increases in the photoperiod are detected, or the accumulation rate of forcing temperature could be accelerated by increasing bud sensitivity to forcing as photoperiod increases, or after passing a certain threshold of photoperiod.

The first statement could explain the behavior observed for the northern parent “58-861”. It was moved from northern to southern latitude suffering a decrease of photoperiod, with a little lack to achieve a photoperiod threshold. Differently, “Poli” was moved from South to North, and the

perception of a longer photoperiod advanced the achievement of forcing an accumulation requirement. Summarizing, all these observations suggest that patterns of genetic variation for bud flush have been modeled by natural selection.

Bud phenological traits here studied were genetically variable and, at least, moderately heritable. With the exception of bf_date2.5, in both years individual heritability (H^2_{ind}) related to onset-of-stages date were very similar. In a breeding context this is an important result, because it suggests as each step was controlled with the same extent by genetic factors. Hence, in order to fix the genetic components in a breeding program, the study of the more easy stages to collect (i.e. bf_date1 and bf_date1.5) should be preferred to inspect the whole process. It is clear that to focus the available resources (i.e. time, money and people) on a limited number of objectives to study allow a greater accuracy of data sampling. Hanley and Karp (2013) underlined how: "In an era of *omic* tools and technologies, it can be easy to overlook the importance of robust phenotypic data, but the value of this could never be over-emphasized".

Unlike the phases, the duration traits showed a major phenotypic variance and minor contribution for the genetic component of the observed variance, as result of a high influence of environmental conditions on these traits (Fabbrini et al., 2012). The overall mean values of H^2_{ind} among the onset-of-stages were 0.45 in VT 2010 and 0.21 in VT 2012. This decreasing heritabilities were caused by the decrement of CV_G (from 1.49% to 1.13%) and the simultaneous increment of CV_ε (from 1.69% to 2.15%). In other words, heritabilities were observed at similar levels of expressed genetic variation in both years (1.49% in VT 2010 and 1.69% in VT 2012). These results remarked that, despite a not negligible micro-environmental influence, bud flush traits are under a strong genetic control (Bradshaw and Stettler 1995). At the same time, greater values of residual variation could be explained by greater rates of mortality occurred in 2012, by a different accuracy of measures and clearly by a different environmental condition between years. H^2_{ind} observed in this work were lower than values observed in other papers. However, the large number of previous works used inter-specific hybrids, that could increase the genetic variance and heritability estimation for phenological traits (Howe et al., 2000). For bf_date1.5, in *P. deltoides* × *P. nigra* and in *P. deltoides* × *P. trichocarpa* crosses, H^2_{ind} greater than 0.65 were observed (Marron et al., 2010b) in trials established in Cavallermaggiore (Italy) and Ardon (France). Moreover, Howe and coworker (2000) observed H^2_{ind} of 0.80 for bf_date2 in a *P. deltoides* × *P. trichocarpa* cross, grew in Oregon (USA). Differently, Olson and coworkers (2013) studied the adaptive potential of natural populations of *Populus balsamifera* in northern America. They observed for bf_date1 values of H^2_{ind} closed to 0.5, similar to the values observed for the POP5 family.

Compared to other sources of variation, very weak genotype by environment interaction was found between years (VT 2010 and VT 2012) for the onset-of-stage date. The very low value of relative component ($\sigma^2_{G\times E}/\sigma^2_P$ =1.30%) observed for bf_date1.5 expressed as DOY suggests that, despite the great influence exercised by climate condition, a consistent response of POP5 family was conserved during the bud flush process. Moreover, high values of Spearman's coefficient (at least 0.56) observed from bf_date0.5 to bf_date2 stages, highlights the lack of random changes of genotype performances. Hence, there was a no plastic behavior of a large number of F_1 genotypes. Otherwise, great relative importance on the total phenotypic variance was observed for the environmental component (σ^2_E/σ^2_P) for DOY, CAT_{10} and CMT_5 parameters. For the five onset-of-stage date expressed in DOY, similar values of σ^2_E/σ^2_P were observed due to the fixed temporal gap between year 2010 and year 2012. Otherwise, different increment of CAT_{10} (greater in VT 2012 than in VT 2010) correspond to increased values of σ^2_E/σ^2_P from bf_date0.5 (47.13%) to bf_date2.5 (96.35%). Moreover, the decreasing of σ^2_G/σ^2_P from bf_date0.5 (16.94%) to bf_date2.5 (0.8%) was observed, due to the decreasing of genotype variance observed in VT 2010 for the onset-of-stage expressed in CAT_{10}. A different pattern was observed for CMT_5 with the total phenotypic variance (99.9%) explained by environmental components. This result is due to the difference of more than 100°C observed between years 2010 and 2012 for CMT_5 and by the low variance of this trait observed during the bud flush phases (in particular in VT 2012). To our knowledge, there are not similar studies concerning the relative contribution of each developing stage through an in-depth analysis of the bud flush process. However, Fabbrini and coworker (2012) set a similar study to explain phenotypic variance of the bud set process. They found that σ^2_G/σ^2_P and $\sigma^2_{G\times E}/\sigma^2_P$ explain at least 30% of phenotypic variance, and more than 9.7% of variance was explained by σ^2_E/σ^2_P. Hence, the genetic variability for bud set was larger than for bud flush, and this could be explained with the more site-specific control exercised by the photoperiod on bud set (Howe et al., 2000).

Phenology traits have a quantitative genetic background and thus QTL mapping is a powerful method to identify genomic regions controlling these traits. In the face of climate change, the main focus of QTL mapping studies in poplars has been on traits tightly linked to environmental adaptation, such as bud blush (Frewen et al., 2000; Chen et al., 2002). In the present study a large number of QTLs were detected for the three central stages of the bud flush process. As observed for biomass traits, the onset-of-stages dates showed high values of phenotypic correlations and collocated QTLs on the same groups. Moreover, it was often observed the collocation of QTLs related to the same stages and expressed both for CAT_{10} and CMT_5. These results suggest as the

detected genomic regions exert a large control on bud flush process, and as temperatures are the principal drivers for genes located inside these regions. However, the large number of genomic regions found on the two parental maps suggest a great polygenic control. Marron and coworkers (2012b) confirmed these results. They found 19 QTL for bf_date1 on LG II, V, VI, VIII, XI, XIV, XVI, XVII, XVIII, XIX on two interspecific poplar families. Moreover, half of the QTLs found showed a 95% confidence intervals wide such as the whole length of linkage group, pointing out the "wide" nature of the genetic control of phenological traits. In the present work, the linkage groups I and VIII showed a particular interest. On LG I was found a large number of QTLs both on maternal and paternal map, near the marker PMGC_2385. Moreover, multi-environment analyses (VT 2010 vs. VT 2012 and CV 2004 vs .MT 2004) provided QTLs with aligned peaks on this LG. A better result occurred for LG VIII, with the QTL peaks (of 8 QTLs) aligned in a range of 20 cM (between markers PMGC_ 2610 and ORPM_264). These results provided a good basis to check "inside" the QTLs common candidate genes of interest involved in the control of the bud flush process.

Concerning phenotypic variance, the mean value of PVE for individual QTL was in the range 8.7% and 23.5%, with an overall mean value among traits of 13.6%. This mean value was greater than mean values observed by Marron and coworkers (2012b) (lower than 7%) and Ghelardini and coworkers (2014) (lower than 3.5%). However, the maximum value of total PVE recorded was of 52% for bf_date1.5 (CMT_5), with an overall mean value of total phenotypic expressed variance for a trait of 35%. The residual 65% of variance remained unexplained by undetected QTLs. Hence, the large number of QTLs found, characterized by small or modest effects, highlights the multi-genetic basis of bud flush phenology.

Our study contributes to improve the knowledge of the genomic regions involved in biomass allocation, allometric traits and springtime bud phenology in black poplar. The analysis of the same traits in different environments and years provide an increased statistical power to detect QTLs and a major accuracy of the estimates of QTLs position and effect (Jansen et al., 1995; Rae et al., 2008). The advent of *Populus trichocarpa* whole genome sequences has opened the possibility of anchoring genetic maps and positioning QTL on a physical map, in order to dissect the *loci* and to find the causative genes of phenotypic variation for biomass and bud flush traits.

6. CONCLUSIONS

Although our results might be specific to the POP5 full-sib family for the studied experimental sites, some interesting general conclusions can be drawn for traits related to aboveground biomass production and bud flush phenology.

6.1. Aboveground biomass production

- Equations established for each environment were adequate to catch the genetic variation available within the POP5 family, with a predominant role of stem circumference in the biomass estimation. Differently, the number of sylleptic branches was not significant in the estimation models, despite the physiological key-role of tree canopy involved in the aboveground biomass determination.
- Standardization between different estimation models was not simple to apply. Between different locations, regardless the difference in the silvicultural managements used in the trial plantations, the "unfillable gaps" observed between regression models pointed out the significant variances between site effects (soil fertility and climate regime). Moreover, standardization observed between different growing seasons in the same site suggested similar biomass productivity between the first and the second rotation.
- Compared to growth performances of selected hybrid poplars, the POP5 family showed considerable mean productivity (11.3 t ha^{-1} $year^{-1}$) in the different sites.
- Southern parent "Poli" showed low adaptability to the different environments. Low survival rates and biomass productions observed in all trials pointed out to the low adaptation ability to continental conditions and coppice regime. Differently, northern parent "58-861" showed high adaptability, with growth performances close to the mean biomass production of the POP5 family in all the environments. In this context, it was clear as "58-861" had greater advantages than "Poli" in temperate, fertile and wet environments, whereas "Poli" showed to be more adapted than "58-861" in warm and dry locations.
- Despite the different canopy architecture of parents, a general pattern of biomass allocation (35% branches – 75% stem) was confirmed by "Poli", "58-861" and POP5 family mean in different environments. In order to maintain this stable equilibrium between stem and branch biomass proportion, the number of sylleptic branches changed in response to different environmental inputs. In fact, the number of sylleptic branches is known as a very

plastic traits and the interaction between genotype and environment is in general significant for branch and canopy traits (Rae et al., 2008).

- POP5 family showed to be a productive group of stable genotypes for biomass production. For this trait low values of G×E relative variance component, without changes in relative position among genotypes, and a few number of plastic genotypes supported this statement.
- Coppicing of the POP5 experimental plantation had these principal effects: (i) to improve the aboveground biomass production in the sites where coppicing was performed at the end of first growing season, (ii) to decrease, with consecutive cuts, the phenotypic variance of percentage of biomass allocated in branches, (iii) to decrease the statistical significance and the extent of genotype by environment interaction (G×E).
- The large number of QTLs mapped for traits determinant the biomass production (circumference, height and total dry mass), each one characterized by small or modest effect, highlights the complex nature of these traits. However, a not negligible number of QTLs with PVE greater than 10%, and a low number of QTLs detected for each trait suggest the possibility of "oligogenic model" control (i.e. a few number of QTLs with moderate values of PVE). The multi-environment analysis approach was a powerful tool for QTLs investigation, that will provide useful information (i.e. common molecular markers between maps) in the future to identify candidate genes in QTL hot spots through comparative mapping with the genome of *Populus trichocarpa*.

6.2. Bud flush phenology

- The protocol proposed to study springtime bud phenology offered the possibility of dissecting bud flush into stages and durations, and to estimate their relative contribution. Moreover, the utilization of thermal parameters provided results related to temperature trend and its effect during the bud flush process. Furthermore, the choice of a starting day of the thermal accumulation based on phenological data from previous investigations on the POP5 family, rather than an arbitrary choice, allowed an accurate characterization of the process. These are the major improvement compared to the previous studies.
- Temperature, as an highly variable parameter among sites and years, was the crucial factor in the onset of growth initiation in poplar as showed by the variation of the pedigree mean between environments.
- The calculation of CAT_{10} parameter was found to be of particular interest to explain the bud flush trigger. In fact, in the two growing seasons the same CAT_{10} values were observed for

the onset of the bud flush. Moreover, after the reaching of this critical heat sum threshold, POP5 family quickly started the process after 3-4 days.

- Low temperature accumulation (i.e. CMT_5) affected the duration of the stages, as highlighted by a great phenotypic variance observed in 2010 than 2012. Instead, the role of CMT_5 on bud flush onset was apparently irrelevant, due to a difference of 100°C between the two growing season.
- Stages related to second part of the process resulted to be more vulnerable to decreasing temperature.
- Southern parent "Poli" showed greater vulnerability to temperature compared to the northern parent "58-861". "Poli" showed to be very responsive to CAT_{10} threshold stopping its growth activity with low temperature during the bud flush process. This evidence pointed out its strong dependence of optimal temperature for growth. Differently, "58-861" required more time to reach the threshold temperature to initiate the bud flush process and was not sensible to low temperature occurred during the bud flush phenology process. However, a secondary role of photoperiod and chilling requirement fulfillment cannot be excluded, in particular to explain effects due to moving parents from the original site conditions.
- Medium values of heritability (H^2=0.50) and high phenotypic correlation among subsequent stages (i.e. "cascade" fashion) suggested as each step was controlled with the same extent by genetic factors, and this is an important result in a breeding context.
- Very weak G×E interaction variance component was found between years for all the bud flush stages, with lack of random changes in genotype ranks and few genotype with high values of relative ecovalence. Hence, despite the great influence exercised by climate conditions, a consistent response of POP5 family was conserved during the time.
- As expected, the large number of overlapped QTLs suggested as the different stages were under the control of the same genomic regions. Moreover, each QTL was characterized by small or modest effect, highlighting the polygenic nature of these traits. The low to moderate total percentage of phenotypic variance explained by QTLs for each trait and year, suggests that there are many QTLs of small effect contributing to the phenotypic variation still undetected.

7. BIBLIOGRAPHY

- Aitchison E.W., Kelley S.L., Alvarez P. J. J., Schnoor J. L., 2000. Phytoremediation of 1,4-dioxane by hybrid poplar trees. ProQuest Science Journal, 72:313-321.

- Annichiarico P., 2002. Genotype x Environment Interactions - Challenges and Opportunities for Plant Breeding and Cultivar Recommendations. FAO plant production and protection paper, n. 174.

- Armstrong A., Johns C., Tubby I., 1999. Effects of spacing and cutting cycle on the yield of poplar grown as an energy crop. Biomass and Bioenergy, 17:305-314.

- Arnold C.Y., 1959. The development and significance of the base temperature in a linear heat unit system. Proceedings of the American Society for Horticultural Science, 74:430-445.

- Baker D. G., Sharratt B. S., Chiang H. C., Zandlo J. A., Ruschy D. L., 1984. Base temperature selection for the prediction of european corn borer instars by the growing degree day method. Agricultural and Forest Meteorology, 32:55-60.

- Barcaccia G., Falcinelli M., 2005. Genetica e genomica. Vol. II. Miglioramento genetico. Liguori Editore, 2005.

- Barsoum N., 2001. Regeneration - requirements and promotion measures. EUFORGEN Technical Bulletin: In situ conservation of *Populus nigra*. International Plant Genetic Resources Institute (IPGRI), Rome, Italy.

- Beaubien E. G. and Freeland H. J., 2000. Spring phenology events in Alberta, Canada: links to ocean temperature. International Journal of Biometeorology, 44:53-59.

- Bedeneau M., Auclair D., 1989. Effect of coppicing on hybrid poplar fine root dynamics. Annales des Sciences Forestieres, 46 (suppl.): 294-296.

- Bernardo R., 2008. Molecular markers and selection for complex traits in plants: learning from the last 20 years. Crop Science, 48:1649-1664.

- Boer M. P., Wright D., Feng L., Podlich D. W., Luo L., Cooper M., van Eeuwijk F. A., 2007. A Mixed-Model Quantitative Trait Loci (QTL) Analysis for Multiple-Environment Trial Data Using Environmental Covariables for QTL-by-Environment Interactions, With an Example in Maize. Genetics, 177:1801-1813.

Box G. E. P. and Cox D. R., 1964. An Analysis of Transformations. Journal of the Royal Statistical Society, Series B (Methodological), 26:211-252.

Braatne J. H., Rood S. B., Heilman P. E., 1996. Life history, ecology and conservation of riparian cottonwoods in North America. In: "Biology of *Populus* and its Implications for Management and Conservation". NRC Research Press, National Research Council of Canada, Ottawa, Canada, 57-85.

Bradshaw H. D. Jr. and Stettler R. F., 1995. Molecular genetics of growth and development in *populus*. IV. Mapping QTLs with large effects on growth, form, and phenology traits in a forest tree. Genetics, 139:963-973.

Bradshaw H. D., Jr., Ceulemans R., Davis J., Stettler R., 2000. Emerging model systems in plant biology: poplar (*Populus*) as a model forest tree. Journal of Plant Growth Regulation, 19:306-313.

Brancourt-Hulmel M., Biarnès-Dumoulin V., Denis J.B., 1997. Guiding marks on stability and genotype-environment interaction in plant breeding. Agronomie, 17:219-246.

Brissette J. C. and Barnes B. V., 1984. Comparisons of phenology and growth of Michigan and western North American sources of *Populus tremuloides*. Canadian Journal of Forest Research, 14:789-793.

Brunner A. M., Busov V. B., Strauss S. H., 2004. Poplar genome sequence: Functional genomics in an ecologically dominant plant species. Trends in Plant Science, 9:49-56.

Burken G. J. and Schnoor J. L., 1999. Distribution and volatilization of organic compounds following uptake by hybrid poplar trees. International Journal of Phytoremediation, Volume 1(2):139-151.

Campbell R. K. and Sugano A. I., 1979. Genecology of bud-burst phenology in Douglas-fir: response to flushing temperature and chilling. Botanical Gazzette, 140:223-231.

Cannel M. G. R. and Smith R. I., 1983. Thermal time, chill days and prediction of budburst in *Picea sitchensis*. Journal of applied Ecology, 20:951-963.

Cannel M. G., 1990. Modelling the phenology of trees. In Modelling to Understand Forest Functions. University of Joensuu, 11-27.

Castellani E., Freccero V., Lapietra G., 1967. Proposta di una scala di differenziazione delle gemme fogliari del pioppo utile per gli interventi antiparassitari. Giornale Botanico Italiano, 101:355-360.

Cervera, M. T., Storme V., Soto A., Ivens B., Van Montagu M., Rajora O. P., Boerjan W., 2005. Intraspecific and interspecific genetic and phylogenetic relationships in the genus *Populus* based on AFLP markers. Theoretical and Applied Genetics, 111:1440-1456.

Ceulemans R., Stettler R. F., Hinckley T. M., Isebrands J. G., Heilman P. E., 1990. Crown architecture of *Populus* clones as determined by branch orientation and branch characteristics. Tree Physiology, 7:157-167.

Coaloa D. and Nervo G., 2011. Poplar wood production in Europe on account of market criticalities and agricultural, forestry and energy policy. Tercer Congreso Internacional de Salicáceas en Argentina.

Chaney R. L., 1983. Plant uptake of inorganic waste constituents. In Land Treatment of Hazardous Wastes. Noyes Data Corp., Park Ridge, pp. 50–76.

Chang S., Lee S., Je C., 2005. Phytoremediation of atrazine by poplar trees: Toxicity, uptake, and transformation. Journal of Environmental Science and Health, Part B: Pesticides, Food Contaminants, and Agricultural Wastes. Volume 40:801-811.

Chen T. H. H., Howe G. T., Bradshaw H. D., 2002. Molecular genetic analysis of dormancy-related traits in poplars. Weed Science Society of America, 50:232-240.

Chen X., 2003. East Asia. In Phenology: An Integrative Environmental Science, pp. 11-25.

Chuine I., Yiou P., Viovy N., Seguin B., Daux V., Ladurie E. L. R., 2004. Grape ripening as a past climate indicator. Nature, 432:289-290.

Cleveland W.S., Grosse E., Shyu W.M., 1992: Local regression models. In Statistical models in S. Chapman and Hall, pp .309-376.

Cocozza C., Cherubini P., Regier N., Saurer M., Frey B., Tognetti R., 2010. Early effects of water deficit on two parental clones of *Populus nigra* grown under different environmental conditions. Functional Plant Biology, 37:244-254.

Costes E. and Guedon Y., 2002. Modelling branching patterns on 1-year-old trunks of six apple cultivars. Annals of Botany, 89:513-524.

Crawley M. J., 2005. Statistics: An Introduction using R. Wiley.

DAFNE, Department of Agriculture, Forests, Nature and Energy. Provided meteorological data for Viterbo (VT).

Deinlein U., Stephan A. B., Horie T., Luo W., Xu G., Schroeder J. I., 2014. Plant salt-tolerance mechanisms. Trends in Plant Science (article in press):1-9.

Dickmann D. I., 2001. The science of poplar culture. In “Poplar culture in north America”. NRC Research Press, pp. 293-308.

Dickmann D. I., Kuzovkina J., 2008. Poplars and Willows of the World, with Emphasis on Silviculturally Important Species. Rome, Italy: FAO Forest Management Division Working Paper IPC/9-2., pp. 129.

Dillen S. Y., Marron N., Bastien C., Ricciotti L., Salani F., Sabatti M., Pinel M. C. P., Rae A. M., Taylor G., Ceulemans R., 2007. Effects of environment and progeny on biomass estimations of five hybrid poplar families grown at three contrasting sites across Europe. Forest Ecology and Management, 252:12-23.

Dillen S. Y., Marron N., Sabatti M., Ceulemans R., Bastien C., 2009. Relationships among productivity determinants in two hybrid poplar families grown during three years at two contrasting sites. Tree Physiology, 29:975–987.

Duckson D. W. Jr., 1987. Climatology. Encyclopedia of Earth Science, pp. 364-365.

Eckenwalder J. E., 1996. Systematics and evolution of Populus. In: “Biology of Populus and its Implications for Management and Conservation”. NRC Research Press, National Research Council of Canada, Ottawa, Canada, pp. 7-32.

Eckenwalder J. E., 2001. Descriptions of clonal characteristics. In: “Poplar Culture in North America“. Part B. Chapter 13. NRC Research Press, National Research Council of Canada, Ottawa, Canada, pp. 328–336.

El-Soda M., Malosetti M., Zwaan B. J., Koornneef M., Aarts M. G. M., 2014. Genotype × environment interaction QTL mapping in plants: lessons from Arabidopsis. Trends in Plant Science, in press:1-9.

Evers B. J., van der Krol A. R., Vos J., Struik P. C., 2011. Understanding shoot branching by modelling form and function. Trends in Plant Science, 9:464-467.

Fabbrini F., Gaudet M., Bastien C., Zaina G., Harfouche A., Beritognolo I., Marron N., Morgante M., Scarascia Mugnozza G., Sabatti M., 2012. Phenotypic plasticity, QTL mapping and genomic characterization of bud set in black poplar. BMC Plant Biology, pp. 12-47.

Falconer D. S. and Mackay, T. F. C., 1996. Introduction to quantitative genetics. 4th ed. Longman Group, 1996.

Fang S., Xue J., Tang L., 2007. Biomass production and carbon sequestration potential in poplar plantations with different management patterns. Journal of Environmental Management, 85:672–679.

FAO, 2008. Synthesis of Country Progress Reports. 23rd Session of the International Poplar Commission. International Poplar Commission, Working Paper IPC/6. Forest Management Division, FAO, Rome (unpublished).

Farmer R. E., Pitcher J.A., 1981. Pollen handling for Southern hardwoods. In: "Pollen Management Handbook". Agriculture Handbook 587. USDA Forest Service, Washington, DC, USA, pp. 77-83.

Farmer R. E. and Reinholt R. W., 1986. Genetic variation in dormancy relations of balsam poplar along a latitudinal transect in north-western Ontario. Silvae Genetica 35:38-42.

Frewen B. E., Chen T. H. H., Howe G. T., Davis J., Rohde A., Boerjan W., Bradshaw H. D. Jr., 2000. Quantitative trait loci and candidate gene mapping of bud set and bud flush in *Populus*. Genetics, 154: 837-845.

Fuchigami L. H., Weiser C. J., Kobayashi K., Timmis R., Gusta L. V., 1982. A degree growth stage (°GS) model and cold acclimation in temperate woody plants. In Plant Cold Hardiness and Freezing Stress. Mechanisms and Crop Implications. Academic Press, pp. 93-116.

Gauch H. G., 1992. Statistical analysis of regional yield trials: AMMI and related analyses of factorial designs. Elsevier, Amsterdam.

Gaudet M., Jorge V., Paolucci I., Beritognolo I., Scarascia Mugnozza G., Sabatti M., 2008. Genetic linkage maps of *Populus nigra* L. including AFLPs, SSRs, SNPs, and sex trait. Tree Genetics and Genomes, 4:25-36.

Gaudet M., Pietrini F., Beritognolo I., Iori V., Zacchini M., Massacci A., Scarascia Mugnozza G., Sabatti M., 2011. Intraspecific variation of physiological and molecular response to cadmium stress in *Populus nigra* L. Tree Physiology, 31:1309-1318.

Gellini R., and Grossoni P., 1997. "Botanica forestale", Vol. II Angiosperme, CEDAM. Padova.

Ghelardini L., Berlin S., Weih M., Lagercrantz U., Gyllenstrand N., Rönnberg-Wästljung A. C., 2014. Genetic architecture of spring and autumn phenology in *Salix*. BMC Plant Biology, 14:31.

Gilmore E. C. and Roger G. S., 1958. Heat units as a method of measuring maturity in corn. Agronomy Journal, 50:611-615.

Granhus A., FlØistad I. S., SØgaard G., 2009. Bud burst timing in *Picea abies* seedlings as affected by temperature during dormancy induction and mild spells during chilling. Tree Physiology, 29:497-503.

Grattapaglia D., Plomion C., Kirst M., Sederoff R. R., 2009. Genomics of growth traits in forest trees. Plant Biology, 12:148-156.

Hamzeh M. and Dayanandan S., 2004. Phylogeny of *Populus* (Salicaceae) based on nucleotide sequences of chloroplast TRNT-TRNF region and nuclear RDNA. American Journal of Botany, 91:1398-1408.

Hanley S. J. and Karp A., 2013. Genetic strategies for dissecting complex traits in biomass willows (*Salix* spp.). Tree Physiology, 00:1-14.

Hänninen H. and Tanino K., 2011. Tree seasonality in a warming climate. Trends in Plant Science, 16:412-416.

Harrington R., Woiwod I., Sparks T., 1999. Climate change and trophic interactions. Trends in Ecology & Evolution, 14:146-150.

Heide O. M., 2003. High autumn temperature delays spring bud burst in boreal trees, counterbalancing the effect of climatic warning. Tree Physiology, 23:931-936.

Horvath D. P., Anderson J. V., Chao W. S., Foley M. E., 2003. Knowing when to grow: Signals regulating bud dormancy. Trends in Plant Science, 8:534-540.

Howe G. T., Saruul P., Davis J., Chen T. H. H., 2000. Quantitative genetics of bud phenology, frost damage, and winter survival in an F_2 family of hybrid poplar. Theoretical and Applied Genetics, 101:632-642.

Howe G. T., Aitken S. N., Neale D. B., Jermstad K. D., Wheeler N. C., Chen T. H. H., 2003. From genotype to phenotype: Unraveling the complexities of cold adaptation in forest trees. Canadian Journal of Botany, 81:1247-1266.

Hughes F. M. R., Adams W. M., Muller E., Nilsson C., Richards K. S. R., Barsoum N., Decamps H., Foussadier R., Girel J., Guilloy H., Hayes A., Johansson M., Lambs L., Pautou G., Peiry J., Perrow M., Vautier F., Winfield M., 2001. The importance of different scale processes for the restoration of floodplain woodlands. Regulated Rivers: Research and Management, 17:325–345.

IFNC, 2009. Inventario Nazionale delle Foreste e dei Serbatoi Forestali di Carbonio. I caratteri quantitative - Parte 1, versione 2. Le stime di superficie 2005. Corpo Forestale dello Stato, Ispettorato generale. CRA-MPF-Trento. pp.178.

Isebrands J. G. and Karnosky D. F., 2001. Environmental benefits of poplar culture In: “Poplar culture in north America”. NRC Research Press, National Research Council of Canada, Ottawa, Canada, pp. 1-42.

Jansen R.C., Van Ooijen J.W., Stam P., Lister C., Dean C., 1995. Genotype-by-environment interaction in genetic mapping of multiple quantitative trait loci. Theoretical and Applied Genetics 9:33-37.

Jansson S. and Douglas C. J., 2007. *Populus*: A Model System for Plant Biology. Annual Review of Plant Biology, 58:435-58.

Juntilla O., 1989. Physiological responses to low temperature. Annales des Sciences Forestières, 46:604-613.

Kao C. H., Zeng Z., Teasdale, R. D., 1999. Multiple interval mapping for quantitative trait loci. Genetics, 152:1203-1216.

Ketterings Q. M., Coe R., van Noordwijk M., Ambagau Y., Palm C. A., 2001. Reducing uncertainty in the use of allometric biomass equations for predicting above-ground tree biomass in mixed secondary forests. Forest Ecology and Management, 146:199-209.

King J. S., Pregitzer K. S., Zak D. R., 1999. Clonal variation in above- and below-ground growth responses of *Populus tremuloides* Michaux: Influence of soil warming and nutrient availability. Plant and Soil, 217:119-130.

Körner C. and Basier D., 2010. Phenology under global warming. Science, 327:1461-1462.

Kramer K., 1996. Phenology and growth of European trees in relation to climate change. PhD thesis, pp. 210.

Kramer K., Leinonen I., Loustau D., 2000. The importance of phenology for the evaluation of impact of climate change on growth of boreal, temperate and Mediterranean forests ecosystems: an overview. International Journal of Biometeorology, 44:67-75.

Kurz W.A., Dymond C.C., Stinson G., Rampley G.J., Neilson E.T., Carroll A.L., Ebata T., Safranyik L., 2008. Mountain pine beetle and forest carbon feedback to climate change. Nature, 452:987-990.

Kuser J. E. and Ching K. K., 1980. Provenance variation in phenology and cold hardiness of western hemlock seedlings. Forest Science, 26:463-470.

Lackner K.S., 2003. A guide to CO_2 sequestration. Science, 300:1677-1678.

Lander E. S. and Botstein D., 1989. Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics,121:185-199.

Laureysens I., Deraedt W., Indeherberge T., Ceulemans R., 2003. Population dynamics in a 6-year old coppice culture of poplar. I. Clonal differences in stool mortality, shoot dynamics and shoot diameter distribution in relation to biomass production. Biomass and Bioenergy, 24:81-95.

Laureysens I., Bogaert J., Blust R., Ceulemans R., 2004. Biomass production of 17 poplar clones in a short-rotation coppice culture on a waste disposal site and its relation to soil characteristics. Forest Ecology and Management, 187:295-309.

Lefèvre F., Legionnet A., de Vries S., Turok J., 1998. Strategies for the conservation of a pioneer tree species, *Populus nigra* L., in Europe. Genetics Selection Evolution, 30: 18-196.

Leinonen I. and Kramer K., 2002. Applications of phenological models to predict the future carbon sequestration potential of boreal forests. Climatic Change, 55:99-113.

Lenihan J.M., Bachelet D., Neilson R.P., Drapek R., 2008. Response of vegetation distribution, ecosystem productivity, and fire to climate change scenarios for California. Climatic Change, 87 (Suppl 1):215-230.

Leyser O., 2009. The control of shoot branching: an example of plant information processing. Plant, Cell & Environment, 6:694-703.

Linkosalo T., Häkkinen R., Terhivuo J., Tuomenvirta H., Hari P., 2009. The time series of flowering and leaf bud burst of boreal trees (1846-2005) support the direct temperature observations of climatic warming. Agricultural and Forest Meteorology, 149:453-461.

Luquez V., Hall D., Albrectsen B. R., Karlsson J., Ingvarsson P., Jansson S., 2008. Natural phenological variation in aspen (*Populus tremula*): the SwAsp collection. Tree Genetics & Genomes, 4:279-292.

Lynch M. and Walsh B., 1998. Genetic and analysis of quantitative traits. Sinauer Associates.

Malosetti M., Ribaut J., van Eeuwijk F. A., 2013. The statistical analysis of multi-environment data: modeling genotype-by-environment interaction and its genetic basis. Frontiers in Physiology, 4:44.

Marron N., Bastien C., Sabatti M., Taylor G., Ceulemans R., 2006. Plasticity of growth and sylleptic branchiness in two poplar families grown at three contrasting sites across Europe. Tree Physiology, 26:935-946.

Marron N., Ricciotti L., Bastien C., Beritognolo I., Gaudet M., Paolucci I., Fabbrini F., Salani F., Dillen S. Y., Ceulemans R., Pinel M. P. C., Taylor G., Scarascia Mugnozza G., Sabatti M., 2010a. Plasticity of growth and biomass production of an intraspecific *Populus alba* family grown at three sites across Europe during three growing seasons. Canadian Journal of Forest Research, 40:1887-1903.

Marron N., Storme V., Dillen S. Y., Bastien C., Ricciotti L., Salani F., Sabatti M., Rae A. M., Ceulemans R., Boerjan W., 2010b. Genomic regions involved in productivity of two interspecific poplar families in Europe. 2. Biomass production and its relationships with tree architecture and phenology. Tree Genetics & Genomes, 6:533-654.

Menzel A., Sparks T. H., Estrella N., Koch E., Aasa A., Ahas R., Alm-kübler K., Bissolli P., Braslavská O., Briede A., Chmielewski F. M., Crepinsek Z., Curnel Y., Dahl A., Defila C., Donnelly A., Filella Y., Jatczak K., Mage F., Mestre A., Nordli Ø., Peñuelas J., Pirinen P., Remišová V., Scheifinger H., Striz M., Susnik A., Van Vliet A. J. H., Wielgolaski F., Zach S., Zust A., 2006. European phenological response to climate change matches the warming pattern. Global Change Biology, 12:1969-1976.

Miles C. M. and Wayne M., 2008. Quantitative Trait Locus (QTL) Analysis. Nature Education 1(1).

Monclus R., Leplé J.C., Bastien C., Bert P.F., Villar M., Marron N., Brignolas F., Jorge V., 2012. Integrating genome annotation and QTL position to identify candidate genes for productivity, architecture and water-use efficiency in *Populus* spp. BMC Plant Biology, 12:173.

Morin X., Viner D., Chuine I., 2008. Tree species range shifts at a continental scale: new predictive insights from a process-based model. Journal of Ecology, 96:784-794.

Morin X., Roy J., Sonié L., Chuine I., 2010. Changes in leaf phenology of three European oak species in response to experimental climate change. New Phytologist, 186:900-910.

Muchero W., Sewell M. M., Ranjan P., Gunter L. E., Tschaplinski T. J., Yin T., Tuskan G. A., 2013. Genome Anchored QTLs for Biomass Productivity in Hybrid Populus Grown under Contrasting Environments. Plos One, DOI: 10.1371/journal.pone.0054468.

Murray M. B., Cannel M. G. R., Smith R. I., 1989. Date of budburst of fifteen tree species in Britain following climatic warming. Journal of Applied Ecology, 26:693-700.

Nunes-Nesi A., Brito D. S., Inostroza-Blancheteau C., Fernie A. R., Araújo W. L., 2014. The complex role of mitochondrial metabolism in plant aluminum resistance. Trends in Plant Science, (article in press):1-9.

Olson M.O., Levsen N., Soolanayakanahally R.Y., Guy R.D., Schroeder W.R., Keller S.R., Tiffin P., 2013. The adaptive potential of *Populus balsamifera* L. to phenology requirements in a warmer global climate. Molecular Ecology, 22:1214-1230.

O'Reilly C.M., Alin S.R., Plisnier P., Cohen A.S., McKee B.A., 2003. Climate change decreases aquatic ecosystem productivity of Lake Tanganyika, Africa. Nature, 424:766-768.

Pan Y., Birdsey R. A., Fang J., Houghton R., Kauppi P. E., Kurz W. A., Phillips O. L., Shvidenko A., Lewis S. L., Canadell J. G., Ciais P., Jackson R. B., Pacala S. W., David McGuire A. D., Piao S., Rautiainen A., Sitch S., Hayes D., 2011. A large and persistent carbon sink in the world's forests. Science, 333:988-993.

Partanen J., Koski V., Hänninen H., 1998. Effects of photoperiod and temperature on the timing of bud burst in Norway spruce (*Picea abies*). Tree Physiology, 18:811-816.

Pellis A., Laureysens I., Ceulemans R., 2004. Genetic variation of the bud and leaf phenology of seventeen poplar clones in a shot rotation coppice culture. Plant Biology, 6:1-9.

Perini L., Beltrano M. C., Dal Monte G., Espostito S., Caruso T., Motisi A., Marra F. P., Ceccarelli T., 2004. Atlante Agroclimatico – agro climatologia, pedologia, fenologia del territorio italiano. UCEA.

Poorter H. and Nagel O., 2000. The role of biomass allocation in the growth response of plants to different levels of light, CO_2, nutrients and water: a quantitative review. Australian Journal of Plant Physiology, 27:595-607.

Proe M. F., Craig J., Griffiths J., Wilson A., Reid E., 1999. Comparison of biomass production in coppice and single stem woodland management systems on imperfectly drained gley soil in central Scotland. Biomass & Bioenergy, 17:141-151.

Rae A. M., Robinson K. M., Street N. R., Taylor G., 2004. Morphological and physiological traits influencing biomass productivity in short-rotation coppice poplar. Canadian Journal of Forest Research, 54:335-345.

Rae A. M., Pinel M. P. C., Bastien C., Sabatti M., Street N. R., Tucker J., Dixon C., Marron N., Dillen S. Y., Taylor G., 2008. QTL for yield in bioenergy *Populus*: identifying G×E interactions from growth at three contrasting sites. Tree Genetics & Genomes, 4:97-112.

Rae A. M., Street N. R., Robinson K. M., Harris N., Taylor G., 2009. Five QTL hotspots for yield in short rotation coppice bioenergy poplar: The Poplar Biomass Loci. BMC Plant Biology, 9:23.

Regier N., Streb S., Cocozza C., Schaub M., Cherubini P., Zeeman S.C., Frey B., 2009. Drought tolerance of two black poplar (*Populus nigra* L.) clones: contribution of carbohydrates and oxidative stress defence. Plant, Cell & Environment, 32:1724-1736.

Remphrey W. R. and Powell G. R., 1985. Crown architecture of Larix laricina saplings: sylleptic branching on the main stem. Canadian Journal of Botany, 63:1296-1302.

Ritson, P., Sochacki, S., 2003. Measurement and prediction of biomass and carbon content of *Pinus pinaster* trees in farm forestry plantations, southwestern Australia. Forest Ecology and Management, 175:103-117.

Rohde A., Howe G. T., Olsen J. E., Moritz T., Van Montagu M., Junttila O., Boerjan W., 2000. Molecular aspects of bud dormancy in trees. In "Molecular Biology of wood plants", Kluwer academic publishers, 1:89-134.

Rohde A., Storme V., Jorge J., Gaudet M., Vitacolonna N., Fabbrini F., Ruttink T., Zaina G., Marron N., Dillen S., Steenackers M., Sabatti M., Morgante M., Boerjan W., Bastien C., 2011. Bud set in poplar – genetic dissection of a complex trait in natural and hybrid population. New Phytologist, 189:106-121.

Root T. L., Price J. T., Hall K. R., Schneider S. H., Rosenzweig C., Pounds J. A., 2003. Fingerprints of global warming on wild animals and plants. Nature, 421:57-60.

Ruml M., Vuković A., Milatović D., 2010. Evaluation of different methods for determining growing degree-day thresholds in apricot cultivars. International Journal of Biometeorology, 54:411-422.

Ruttink T., Arend M., Morreel K., Storme V., Rombauts S., Fromm J., Bhalerao R. P., Boerjan W., Rohde A., 2007. A molecular timetable for apical bud formation and dormancy induction in poplar. Plant Cell, 19:2370-2390.

Saccheri I.J., Rousset F., Watts P.C., Brakefield P.M., Cook L.M., 2008. Selection and gene flow on a diminishing cline of melanic peppered moths. Proceedings of the National Academy of Sciences of the United States of America, 105:16212-16217.

Scarascia Mugnozza G. E., 1991. Physiological and morphological determinants of yield in intensively cultured poplar (*Populus* spp.). Ph.D. Thesis, University of Washington, Seattle, USA, pp. 189.

Scarascia Mugnozza G. E., Ceulemans R., Heilman P. E., Isebrands J. G., Settler R. F., Hinckley T. M.,1997. Production physiology and morphology of *Populus* species and their hybrids grown under short rotation. II. Biomass components and harvest index of hybrid and parental species clones. Canadian Journal of Forest Research, 27:285-294.

Schork N. J., 1993. Extended multipoint identity-by-descent analysis of human quantitative traits: efficiency, power, and modeling considerations. The American Journal of Human Genetics, 53:1306-1313.

Semagn K. BjØrnstad A., Xu Y., 2010. The genetic dissection of quantitative traits in crops. Electronic Journal of Biotechnology, 13:1-45.

Sims R. E. H., Hastings A., Schlamadinger B., Taylor G., Smith P., 2006. Energy crops: current status and future prospects. Global Change Biology, 12:2459-2471.

Sokal R. R. and Rohlf F. J., 1995. Biometry. The principles and practice of statistics in biological research. W. H. Freeman and Company.

Soliani L., 2005. Manuale di statistica per la ricerca e la professione – Statistica univariata e bivariata, parametrica e non-parametrica per le discipline ambientali e biologiche. Avalilable at: http://www.dsa.unipr.it/soliani/soliani.html.

Soolanayakanahally R. Y., Guy R. D., Silim S. N., Song M., 2013. Timing of photoperiodic competency causes phonological mismatch in balsam poplar (*Populus balsamifera* L.). Plant, Cell & Environment, 36:116-127.

Sozzani R., Busch W., Spalding E. P., Benfey P. N., 2013. Advanced imaging techniques for the study of plant growth and development. Trends in Plant Science, (article in press):1-7.

Stanton B. J., Villar M., 1996. Controlled reproduction of Populus. In: "Biology of *Populus* and its Implications for Management and Conservation". NRC Research Press, National Research Council of Canada, Ottawa, Canada, pp. 113-138.

Stettler R. F., Bradshaw H. D., Jr., 1996. Evolution, Genetics, and Genetic Manipulation. In: "Biology of *Populus* and its Implications for Management and Conservation". NRC Research Press, National Research Council of Canada, Ottawa,Canada, pp. 1-6.

Susarla S., Medina V. F., McCutcheon S. C., 2002. Phytoremediation: An ecological solution to organic chemical contamination. Ecological Engineering, 18:647-658.

Tanino K. K., Kalcsits L., Silim S., Kendall E., Gray G. R., 2010. Temperature-driven plasticity in growth cessation and dormancy development in deciduous woody plants: a working hypothesis suggesting how molecular and cellular function is affected by temperature during dormancy induction. Plant Molecular Biology, 73:49-65.

Thompson P. L., Ramer L. A., Schnoor J. L., 1998. Uptake and transformation of TNT by hybrid poplar trees. Environmental Science & Technology, 32:975-980.

Tsarouhas V., Gullberg U., Lagercrantz U., 2003. Mapping of quantitative trait loci controlling timing of bud flush in *Salix*. Hereditas, 138:172-178.

Tuskan G. A. and Rensema T. R., 1992. Clonal differences in biomass characteristics, coppice ability, and biomass prediction equations among four *Populus* clones grown in eastern North Dakota. Canadian Journal of Forest Research, 22:348-354.

Tuskan G. A., 1998. Short rotation woody crop supply systems in the United States: what do we know and what do we need to know?. Biomass and Bioenergy, 14:307-315.

Tuskan G. A., Di Fazio S., Jansson S., Bohlmann J., Grigoriev I., Hellsten U., Putnam N., Ralph S., Rombauts S., Salamov A., Schein J., Sterck L., Aerts A., Bhalerao R. R., Bhalerao R. P., Blaudez D., Boerjan W., Brun A., Brunner A., Busov V., Campbell M., Carlson J., Chalot M., Chapman J., Chen G. L., Cooper D., Coutinho P. M., Couturier J., Covert S., Cronk Q., Cunningham R., Davis J., Degroeve S., Déjardin A., DePamphilis C., Detter J., Dirks B., Dubchak I., Duplessis S., Ehlting J., Ellis B., Gendler K., Goodstein D., Gribskov M., Grimwood J., Groover A., Gunter L., Hamberger B., Heinze B., Helariutta Y., Henrissat B., Holligan D., Holt R., Huang W., Islam-Faridi N., Jones S., Jones-Rhoades M., Jorgensen R., Joshi C., Kangasjärvi J., Karlsson J., Kelleher C., Kirkpatrick R., Kirst M., Kohler A., Kalluri U., Larimer F., Leebens-Mack J., Leplé J. C., Locascio P., Lou Y., Lucas S., Martin F., Montanini B., Napoli C., Nelson D. R., Nelson C., Nieminen K., Nilsson O., Pereda V., Peter G., Philippe R., Pilate G., Poliakov A., Razumovskaya J., Richardson P., Rinaldi C., Ritland K., Rouzé P., Ryaboy D., Schmutz J., Schrader J., Segerman B., Shin H., Siddiqui A., Sterky F., Terry A., Tsai C.J., Uberbacher E., Unneberg P., Vahala J., Wall K., Wessler S., Yang G., Yin T., Douglas C., Marra M., Sandberg G., Van De Peer Y., Rokhsar D., 2006. The genome of black cottonwood, *Populus trichocarpa* (Torr. & Gray). Science, 313: 1596-1604.

Umina P.A., Weeks A.R., Kearney M.R., McKechnie S.W., Hoffmann A.A., 2005. A rapid shift in a classic clinal pattern in *Drosophila* reflecting climate change. Science, 308:691-693.

Updegraff K, Baughmann M. J., Taff S. J., 2004. Environmental benefits of cropland conversion to hybrid poplar: economic and policy considerations. Biomass and Bioenergy, 27:411-428.

Vanden Broeck A., 2003. EURFORGEN Technical Guidelines for genetic conservation and use for European black poplar (*Populus nigra* L.). International Plant Genetic Resources Institute (IPGRI), Rome, Italy.

van der Schoot C. and Rinne P., 1999. Networks for shoot design. Trends in Plant Science, 1:31-37.

van Hecke P., Moermans R., Mau F., Guittet J., 1995. Border effects and size inequality in experimental even-aged stands of poplar clones (*Populus*). Annales des Sciences Forestieres 52:193-200.

Van Looy K., Severyns J., Jochems H., De Smedt F., 2005. Predicting patterns of riparian forest restoration. Archiv für Hydrobiologie 15 (Suppl. 1–4):373–390.

Verwijst, T. and Telenius B., 1999. Biomass estimation procedures in short rotation forestry. Forest Ecology and Management, 121:137-146.

Vietto L., 2009. Risorse genetiche forestali conservate ex.situ. In: "Risorse Genetiche Forestali in Italia". Edited by F. Ducci, A. De Rogatis. CRA SEL, Arezzo. 4:6-22.

Visscher P. M., Hill W. G., Wray N. R., 2008. Heritability in the genomics era — concepts and misconceptions. Nature Reviews Genetics, 9:255-266.

Visser M. E. and Holleman L. J. M., 2001. Warmer springs disrupt the synchrony of oak and winter moth phenology. Proceedings of the Royal Society B., Biological Sciences, 268:289-294.

Vitasse Y. and Basler D., 2013. What role for photoperiod in the bud burst phenology of European beech. European Journal of Forest Research, 132:1-8.

Walther G. R., Post E., Convey P., Menzel A., Parmesan C., Beebee T. J. C., Fromentin J. M., Guldberg O. H., Bairlein F., 2002. Ecological responses to recent climate change. Nature, 416:389-395.

Wang, J. R. and Kimmins J. P., 2002. Biomass estimation errors associated with the use of published regression equations of paper birch and trembling aspen. Northern Journal of Applied Forestry ,19:128-136.

Weisgerber H., 1999. *Populus nigra* Linné, 1753. Enzyklopädie der Holzgewächse, pp. 16-24.

White T. L., Adams W. T., Neale D. B., 2007. Forest Genetics. CABI Publishing.

Worrall J., 1983. Temperature – bud-burst relationships in amabilis and subalpine fir provenance tests replicated at different elevations. Silvae Genetica, 32:203-209.

Wricke G., 1962. Uber eine Methode zur Erfassung der ökologischen Streubreite in Feldversuchen. Zeitschrift für Pflanzenzüchtung, 47:92-96.

Wyckoff G.W. and Zasada J. C., 2007. *Populus* L. In Woody Plant Seed Manual. Washington, D.C.: U.S.D.A. Forest Service.

Wullschleger S. D., Tuskan G. A., Di Fazio S. P., 2002. Genomics and the tree physiologist. Tree Physiology, 22:1273-1276.

Wullschleger S. D., Yin T. M., Di Fazio S. P., Tschaplinski T. J., Gunter L. E., M.F. Davis M. F., Tuskan G. A., 2005. Phenotypic variation in growth and biomass distribution for two advanced-generation pedigrees of hybrid poplar. Canadian Journal of Forest Research, 35: 1779-1789.

Yang S., Logan J., Coffey D. L., 1995. Mathematical formulae for calculating the base temperature for growing degree days. Agricultural and Forest Meteorology, 74:61-74.

Zabek L. M. and Prescott C. E., 2006. Biomass equations and carbon content of aboveground leafless biomass of hybrid poplar in Coastal British Columbia. Forest Ecology and Management, 223:291-302.

Zacchini M., Iori V., Scarascia Mugnozza G., Pietrini F., Massacci A., 2011. Cadmium accumulation and tolerance in *Populus nigra* and *Salix alba*. Biologia Plantarum, 55:383-386.

Zavitkovski J., 1981. Small plots with unplanted plot border can distort data in biomass production studies. Canadian Journal of Forest Research, 11:9-12.

Zobel B. Z., Talbert J. T., 1984. Applied Forest Tree Improvement. New York: Wiley & Sons.

Zsuffa L., 1975. A summary review of interspecific breeding in the genus *Populus* L. Proceedings 14th meeting of the Canadian Tree Improvement Association, part 2. Dept. Environment, Canadian Forestry Service, Ottawa, pp. 107:123.

8. APPENDIX 1

Table 8.1: Pearson's phenotypic correlations between biomass and bud flush traits for CV 2004 based on genotypic means. Correlation significance is shown as: *ns* = non significant, * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$.

CV 2004

Trait	Syll1	Height2	Circum2	TotalDM	bf_date1.5 (DOY)	bf_date1.5 (CAT_{10})	bf_date1.5 (CMT_5)
Syll1	-						
Height2	0.30 ***	-					
Circum2	0.41 ***	0.80 ***	-				
TotalDM	0.40 ***	0.86 ***	0.98 ***	-			
bf_date1.5 (DOY)	0.07 *ns*	0.13 *ns*	0.06 *ns*	0.04 *ns*	-		
bf_date1.5 (CAT_{10})	0.01 *ns*	0.07 *ns*	0.00 *ns*	-0.01 *ns*	0.89 ***	-	
bf_date1.5 (CMT_5)	0.09 *ns*	0.11 *ns*	0.05 *ns*	0.03 *ns*	0.95 ***	0.72 ***	-

Table 8.2: Pearson's phenotypic correlations between biomass and bud flush traits for MT 2004 based on genotypic means. Correlation significance is shown as: *ns* = non significant, * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$.

MT 2004

Trait	Syll1	Height2	Circum2	TotalDM	bf_date1.5 (DOY)	bf_date1.5 (CAT_{10})	bf_date1.5 (CMT_5)
Syll1	-						
Height2	0.31 *ns*	-					
Circum2	0.35 ***	0.77 ***	-				
TotalDM	0.35 ***	0.73 ***	0.93 ***	-			
bf_date1.5 (DOY)	0.03 *ns*	0.29 **	0.18 *	0.23 *	-		
bf_date1.5 (CAT_{10})	0.00 *ns*	0.26 **	0.17 *ns*	0.22 *	0.90 ***	-	
bf_date1.5 (CMT_5)	0.05 ***	0.30 ***	0.19 *	0.22 *	0.96 ***	0.78 ***	-

Table 8.3: Pearson's phenotypic correlations between biomass and bud flush traits for VT 2010 based on genotypic means. Correlation significance is shown as: *ns* = non significant, * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$.

VT 2010

Trait	Height2	Circum2	TotalDM	bf_date1 (DOY)	bf_date1.5 (DOY)	bf_date2 (DOY)	subproc1 (DOY)
Height2	-						
Circum2	0.97 ***	-					
TotalDM	0.90 ***	0.98 ***	-				
bf_date1 (DOY)	0.07 *ns*	0.09 *ns*	0.09 *ns*	-			
bf_date1.5 (DOY)	0.07 *ns*	0.10 *ns*	0.09 *ns*	0.98 **	-		
bf_date2 (DOY)	-0.01 *ns*	0.01 *ns*	0.01 *ns*	0.91 ***	0.95 ***	-	
subproc1 (DOY)	-0.17 *	-0.16 *	-0.16 *ns*	0.13 *ns*	0.25 **	0.52 ***	-

Table 8.4: Pearson's phenotypic correlations between biomass and bud flush traits for VT 2012 based on genotypic means. Correlation significance is shown as: *ns* = non significant, * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$.

VT 2012

Trait	Height2	Circum2	TotalDM	bf_date1 (DOY)	bf_date1.5 (DOY)	bf_date2 (DOY)	subproc1 (DOY)
Height2	-						
Circum2	0.94 ***	-					
TotalDM	0.87 ***	0.97 ***	-				
bf_date1 (DOY)	0.12 *ns*	0.12 *ns*	0.12 *ns*	-			
bf_date1.5 (DOY)	0.14 *ns*	0.14 *ns*	0.15 *ns*	0.96 ***	-		
bf_date2 (DOY)	0.11 *ns*	0.12 *ns*	0.13 *ns*	0.91 ***	0.98 ***	-	
subproc1 (DOY)	-0.01 *ns*	0.00 *ns*	0.03 *ns*	-0.17 *ns*	0.08 *ns*	0.26 **	-

Table 8.5: Pearson's phenotypic correlations between bud flush traits for VT 2010 based on genotypic means (DOY and CAT_{10} data). Correlation significance is shown as: *ns* = non significant, * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$.

VT 2010 (DOY-CAT_{10})

Trait	bf_date0.5 (DOY)	bf_date1 (DOY)	bf_date1.5 (DOY)	bf_date2 (DOY)	bf_date2.5 (DOY)	duration0.5 (CAT_{10})	duration1 (CAT_{10})	duration1.5 (CAT_{10})	duration2 (CAT_{10})	subproc1 (CAT_{10})
bf_date0.5 (DOY)	-									
bf_date1 (DOY)	0.98 ***	-								
bf_date1.5 (DOY)	0.94 ***	0.98 ***	-							
bf_date2 (DOY)	0.87 ***	0.91 ***	0.95 ***	-						
bf_date2.5 (DOY)	0.69 ***	0.71 ***	0.76 ***	0.90 ***	-					
duration0.5 (CAT_{10})	0.27 ***	0.44 ***	0.52 ***	0.53 ***	0.36 ***	-				
duration1 (CAT_{10})	-0.73 ***	-0.68 ***	-0.59 ***	-0.40 ***	-0.22 **	0.11 *ns*	-			
duration1.5 (CAT_{10})	-0.68 ***	-0.70 ***	-0.65 ***	-0.42 ***	-0.04 *ns*	-0.38 ***	0.62 ***	-		
duration2 (CAT_{10})	-0.35 ***	-0.39 ***	-0.37 ***	-0.18 *	0.19 *	-0.40 ***	0.35 ***	0.76 ***	-	
subproc1 (CAT_{10})	-0.78 ***	-0.77 ***	-0.68 ***	-0.45 ***	-0.14 *ns*	-0.15 *ns*	0.90 ***	0.90 ***	0.62 ***	-

Table 8.6: Pearson's phenotypic correlations between bud flush traits for VT 2010 based on genotypic means (DOY and CMT_5 data). Correlation significance is shown as: *ns* = non significant, * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$.

VT 2010 (DOY-CMT_5)

Trait	bf_date0.5 (DOY)	bf_date1 (DOY)	bf_date1.5 (DOY)	bf_date2 (DOY)	bf_date2.5 (DOY)	duration0.5 (CMT_5)	duration1 (CMT_5)	duration1.5 (CMT_5)	duration2 (CMT_5)	subproc1 (CMT_5)
bf_date0.5 (DOY)	-									
bf_date1 (DOY)	0.98 ***	-								
bf_date1.5 (DOY)	0.94 ***	0.98 ***	-							
bf_date2 (DOY)	0.87 ***	0.91 ***	0.95 ***	-						
bf_date2.5 (DOY)	0.69 ***	0.71 ***	0.76 ***	0.90 ***	-					
duration0.5 (CMT_5)	0.91 ***	0.96 ***	0.98 ***	0.95 ***	0.77 ***	-				
duration1 (CMT_5)	0.52 ***	0.62 ***	0.72 ***	0.83 ***	0.78 ***	0.78 ***	-			
duration1.5 (CMT_5)	-0.42 ***	-0.39 ***	-0.34 ***	-0.05 *ns*	0.27 ***	-0.30 ***	0.18 *	-		
duration2 (CMT_5)	-0.67 ***	-0.70 ***	-0.67 ***	-0.55 ***	-0.22 **	-0.63 ***	-0.33 ***	0.47 ***	-	
subproc1 (CMT_5)	-0.03 *ns*	0.05 *ns*	0.14 *ns*	0.41 ***	0.62 ***	0.20 *	0.68 ***	0.85 ***	0.18 *	-

Table 8.7: Pearson's phenotypic correlations between bud flush traits for VT 2012 based on genotypic means (DOY and CAT_{10} data). Correlation significance is shown as: *ns* = non significant, * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$.

VT 2012 (DOY-CAT_{10})

Trait	bf_date0.5 (DOY)	bf_date1 (DOY)	bf_date1.5 (DOY)	bf_date2 (DOY)	bf_date2.5 (DOY)	duration0.5 (CAT_{10})	duration1 (CAT_{10})	duration1.5 (CAT_{10})	duration2 (CAT_{10})	subproc1 (CAT_{10})
bf_date0.5 (DOY)	-									
bf_date1 (DOY)	0.91 ***	-								
bf_date1.5 (DOY)	0.82 ***	0.96 ***	-							
bf_date2 (DOY)	0.76 ***	0.90 ***	0.98 ***	-						
bf_date2.5 (DOY)	0.67 ***	0.76 ***	0.85 ***	0.90 ***	-					
duration0.5 (CAT_{10})	0.81 ***	0.94 ***	0.91 ***	0.85 ***	0.75 ***	-				
duration1 (CAT_{10})	0.01 *ns*	0.22 *	0.41 ***	0.46 ***	0.36 ***	0.12 *ns*	-			
duration1.5 (CAT_{10})	-0.29 **	-0.35 ***	-0.23 *	-0.08 *ns*	0.20 *	-0.29 **	-0.14 *ns*	-		
duration2 (CAT_{10})	-0.36 ***	-0.51 ***	-0.56 ***	-0.51 ***	-0.22 *	-0.42 ***	-0.61 ***	0.37 ***	-	
subproc1 (CAT_{10})	-0.16 *ns*	0.00 *ns*	0.24 **	0.37 ***	0.44 ***	-0.06 *ns*	0.83 ***	0.44 ***	-0.35 ***	-

Table 8.8: Pearson's phenotypic correlations between bud flush traits for VT 2012 based on genotypic means (DOY and CMT_5 data). Correlation significance is shown as: *ns* = non significant, * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$.

VT 2012 (DOY-CMT_5)

Trait	bf_date0.5 (DOY)	bf_date1 (DOY)	bf_date1.5 (DOY)	bf_date2 (DOY)	bf_date2.5 (DOY)	duration0.5 (CMT_5)	duration1 (CMT_5)	duration1.5 (CMT_5)	duration2 (CMT_5)	subproc1 (CMT_5)
bf_date0.5 (DOY)	-									
bf_date1 (DOY)	0.91 ***	-								
bf_date1.5 (DOY)	0.82 ***	0.96 ***	-							
bf_date2 (DOY)	0.76 ***	0.90 ***	0.98 ***	-						
bf_date2.5 (DOY)	0.67 ***	0.76 ***	0.85 ***	0.90 ***	-					
duration0.5 (CMT_5)	-0.53 ***	-0.24 **	-0.06 *ns*	-0.01 *ns*	-0.03 *ns*	-				
duration1 (CMT_5)	-0.68 ***	-0.60 ***	-0.42 ***	-0.29 **	-0.12 *ns*	0.60 ***	-			
duration1.5 (CMT_5)	-0.80 ***	-0.85 ***	-0.77 ***	-0.66 ***	-0.48 ***	0.18 *ns*	0.70 ***	-		
duration2 (CMT_5)	-0.68 ***	-0.80 ***	-0.87 ***	-0.85 ***	-0.74 ***	0.00 *ns*	0.29 **	0.67 ***	-	
subproc1 (CMT_5)	-0.81 ***	-0.81 ***	-0.67 ***	-0.54 ***	-0.35 ***	0.40 ***	0.90 ***	0.94 ***	0.54 ***	-

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