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Chapter 12
Genetic Variability and Determinism of Adaptation of Plants to Soil Waterlogging

Julien Parelle, Erwin Dreyer, and Oliver Brendel

Abstract Flooding or waterlogging, and associated soil hypoxia, affect severely the growth and fitness of plant species, from crops to forest ecosystems. An improved understanding of the intra-species genetic diversity of traits involved in hypoxia tolerance is a prerequisite for crop breeding programmes aimed at increasing the tolerance to waterlogging, as well as for assessing the adaptability of natural populations to waterlogging. Some genotypes within the species have developed adaptations to hypoxia, as shown by differences among populations in growth and fitness, and in traits conferring some degree of tolerance such as sequence, expression and activity of alcohol dehydrogenase, or the ability to develop adventitious roots, increased tissue porosity and hypertrophied lenticels. Genetic control has been estimated for a number of such traits. Overall, under waterlogging, specific tolerance traits show higher heritabilities compared to traits quantifying productivity, damage or overall performance. Genomic regions involved in the control of these traits (i.e., Quantitative Trait Loci QTL) have been detected for tolerance traits in a few species, and allow gaining some insight into the genetic basis of the observed natural diversity or may be a starting point for breeding purposes. However, only for submergence tolerance in rice (sub-1) has a successful gene candidate approach resulted in the detection of alleles that are directly involved in the tolerance process.

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Abbreviations

ADH	Alcohol dehydrogenase
LEI	Lowest elongated internode
PDC	Pyruvate decarboxylase complex
PEV	Per cent of explained variance
QTL	quantitative trait loci
RIL	Real isogenic lines
SNP	Single nucleotide polymorphism
Sub	Submergence tolerance locus

12.1 Introduction

Excess soil water due to flooding or temporary waterlogging can be a major constraint on growth and yield of crops (Tuberosa and Salvi 2004) and forest stands (Kozlowski 1997). It affects severely growth and probably also fitness and distribution of plant species in natural environments. Some species or genotypes within the plant species have developed adaptive responses to flooding and waterlogging. In the case of crops, the occurrence of some genetic diversity in tolerance traits is a prerequisite for breeding programmes. In natural ecosystems, due to the local occurrence of temporarily waterlogged soils (often called hydromorphic soils, Lévy et al. 1999), the frequency and severity of episodes of waterlogging or flooding act as a selective pressure and differences in tolerance can develop among species, or populations within species. To gain insight into the degree of inter-specific variability, we need a careful quantification of the tolerance to waterlogging in individuals and methods to assess it as objectively as possible.

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Two major situations of excess water can be identified (Colmer and Voesenek 2009). Flooding, the partial, or in some cases the complete submergence of the shoot, can be permanent, such as in mangrove ecosystems, or temporary, such as in floodplains or in rice paddies. Waterlogging, due to excess water in the soil, usually occurs temporarily with a water level below or not much above the soil surface that affects primarily the root system and can occur in natural as well as in cultivated ecosystems, depending on soil type and water table dynamics.

In both cases, a temporal sequence of chemical changes occurs in the soil following the onset of waterlogging or flooding (Setter and Waters 2003). Due to a reduced gas exchange between soil and atmosphere, changes in soil bacteria populations occur, oxygen concentration decreases rapidly (hypoxia), carbon dioxide and ethylene concentrations increase, reduced and toxic cations such as manganese (Mn^{++}) and iron (Fe^{++}) accumulate, and an intense de-nitrification occurs. In case of prolonged waterlogging, soils may be completely depleted of oxygen (anoxia) and hydrogen sulphide and methane are produced and diffuse into the atmosphere. Except the last, all of these steps occur usually within the first 20 days of waterlogging. In some soils, this sequence may even occur faster. In this review, we will concentrate on the hypoxia induced by waterlogging or total submergence with all the consequences it might have on respiration, metabolism and growth of affected plants.

To date, the processes conferring some degree of tolerance to waterlogging and hypoxia are still not fully understood despite accumulating information (e.g., Vartapetian 2006; Voesenek et al. 2006; Colmer and Voesenek 2009; Kawano et al. 2009; Jackson et al. 2009; Parolin 2009). The degree of tolerance to a given level of waterlogging may be assessed: (1) indirectly through damage indices or the observed, usually negative, impact on growth, productivity and survival, or (2) directly by evaluating the occurrence of traits contributing to the acclimation to hypoxia (adaptive traits). These traits can be constitutive (i.e., they occur already in individuals growing under optimal conditions and provide some advantage during waterlogging) or induced (i.e., they appear only during episodes of waterlogging in response to a signalling cascade). Induced traits can be roughly grouped into short-term responses (e.g., metabolic adjustments) and long-term acclimations (e.g., development of aerenchyma). A typical short-term response of roots is a decrease of respiration and an increase of glycolytic flux and alcoholic fermentation (Drew 1997). Some key enzymes in this process are alcohol dehydrogenase (McManmon and Crawford 1971; Chan and Burton 1992; Bailey-Serres and Voesenek 2008), sucrose synthase or hexokinases (Germain et al. 1997; Ricard et al. 1998). Long-term responses are mainly related to growth, either of existing or of newly formed structures. In rice, where total submergence clearly poses a major problem for productivity (Tuberosa and Salvi 2004), the elongation of internodes is an important adaptive trait, resulting either in quiescence or an escape strategy (Bailey-Serres and Voesenek 2008). The quiescence strategy consists in a lack of elongation (Xu and Mackill 1996), whereas the escape strategy consists in an enhanced growth rate that maintains the top of the shoot above the water level (Fukao et al. 2006). Adaptive morphological traits are slower to develop compared to purely physiological or metabolic adjustments. Assessment of such traits requires long-term experiments with the risk of an interaction between ontogenic development and stress response. A few anatomical traits, thought to allow transport of oxygen to roots and enable a partial maintenance of respiration, survival or even root growth, have commonly been measured in experiments on genetic variability. They include the development of hypertrophied lenticels (Parelle et al. 2007), of adventitious roots (Mano et al. 2005a, b) and of aerenchyma (porosity) in root or stem tissues (Zaidi et al. 2007; Mollard et al. 2008; Mano et al. 2007, 2008; Mano and Omori 2008; and see also Chap. 6 in this volume).

From an agronomic point of view, the maintenance of productivity, particularly yield, is of major importance. This can be evaluated by quantifying growth or biomass and also more indirectly by assessing, among others, leaf level gas exchange or photosynthetic capacity. Leaf gas exchange, for instance, has been used (Dreyer 1994; Wagner and Dreyer 1997) to characterise the overall performance under waterlogging. Such traits bring no information about the morphological and physiological mechanisms of tolerance; nevertheless, maintenance of productivity or photosynthesis contributes to fitness and survival of individuals. Survival rate under hypoxia is, together with shoot dieback and other fitness related traits (number of seeds produced, etc), an important means to assess the degree of tolerance of populations. Leaf epinasty, the downward growth of leaf petioles, is a specific response to root hypoxia in some species (Jackson and Campbell 1976) and a direct indicator of the level of hypoxia stress perceived by the individuals (Vartapetian and Jackson 1997). All these traits may respond to waterlogging with quite different intensities. On the other hand, some traits obviously play a direct adaptive role, or at least are thought to do so. Such traits include the development of hypertrophied lenticels, of adventitious roots, of aerenchyma or the occurrence of physiological changes (switch from a respiratory to a fermentative metabolism). All these traits contribute to mitigate the impact of hypoxia in the soil, by maintaining a minimal supply of oxygen to roots.

The quantification of growth decline under stress provides a first indication about the level of tolerance of a genotype. Traits that have been measured include plant height, growth increment and shoot or root dry weight (see Table 12.1 for examples from quantitative genetic studies). Yield, or the reduction thereof, has also been quantified for crops under waterlogging (Vantoai et al. 2001; Githiri

Table 12.1 Traits tested during QTL experiments to identify hypoxia-tolerance related loci in different species

Stress type	Article	Genus	Trait types
Submergence	Xu and Mackill (1996)	Rice	Damage
	Toojinda et al. (2003)	Rice	Damage
	Nandi et al. (1997)	Rice	Survival
	Sripongpangkul et al. (2000)	Rice	Survival
	Toojinda et al. (2003)	Rice	Survival
	Toojinda et al. (2003)	Rice	Growth
	Ikeda et al. (2007)	Rice	Growth
	Nemoto et al. (2004)	Rice	Elongation
	Tang et al. (2005)	Rice	Elongation
	Hattori et al. (2007)	Rice	Elongation
	Sripongpangkul et al. (2000)	Rice	Elongation
	Toojinda et al. (2003)	Rice	Elongation
Waterlogging	Mano et al. (2006)	Maize	Damage
	Cornelious et al. (2005)	Soybean	Damage
	Martin et al. (2006)	Iris	Survival
	Vantoai et al. (2001)	Soybean	Growth
	Parelle et al. (2007)	Oak	Growth
	Qiu et al. (2007)	Maize	Growth
	Vantoai et al. (2001)	Soybean	Yield
	Githiri et al. (2006)	Soybean	Yield
	Mano et al. (2005a)	Maize	Adventitious roots
	Mano et al. (2005b)	Maize	Adventitious roots
	Zheng et al. (2003)	Rice	Adventitious roots
	Parelle et al. (2007)	Oak	Hypertrophied lenticels
Control	Mano et al. (2007, 2008)	Maize	Aerenchyma

et al. 2006). However, the use of these traits to detect genetic differences in waterlogging tolerance requires a careful interpretation to identify adaptive traits involved in the tolerance to hypoxia, in contrast to nonadaptive traits indicating merely a genetic difference, for instance in growth potential.

The quantification of damage induced by waterlogging may also provide an estimation of tolerance. Some authors use visual ordinal scales of damage (Xu and Mackill 1996; Sripongpangkul et al. 2000; Cornelious et al. 2005), others quantify leaf senescence (Toojinda et al. 2003), fraction of yellow leaves (Zhou et al. 2007a) or decline in leaf chlorophyll content (GuangHeng et al. 2006). Damage indices have been successfully used to study the genetic determinism of tolerance in crops. For example, the so-called *sub-1* locus (Xu and Mackill 1996) was identified in rice 10 years before the actual process controlled by the locus was understood (Xu et al. 2006). A large amount of damage eventually leads to mortality, which is a very simple approach to characterising tolerance (Nandi et al. 1997; Sripongpangkul et al. 2000; Toojinda et al. 2003; Martin et al. 2006). Despite the fact that survival is an ordinal trait, the approximately normal distribution within experiments allows using it for a genetic trait dissection in rice (Xu and Mackill 1996) and soybean (Cornelious et al. 2005). The advantage of survival and damage traits is that the variability tested is directly related to the stress tolerance, and can therefore be fully attributed to genetic diversity.

To assess the genetic variability of tolerance to waterlogging or flooding, the most obvious procedure is the quantitative analysis of traits conferring directly or indirectly some level of tolerance to waterlogging. Prerequisites for suitable traits include: (1) the relevance and specificity of the trait as an indicator of adaptation; (2) the repeatability of the measurement procedure and (3) the possibility of assessing a large number of individuals.

Genetic variability of tolerance traits can be studied in situ in natural populations only when detailed information on the environmental conditions and their spatial and temporal variability is available. Further, an already advanced knowledge of the genetic determinism of the trait studied is necessary, with a remaining risk of confusion between purely genetic differences and genotype and environment interactions. Therefore, all of the studies reviewed here were done using common conditions for all genotypes (vegetative copies or half-sib families), as provided by common-garden plantations (comparative plantations with a homogenized environment) or greenhouse experiments. However, even under such controlled conditions, statistical methods (such as complete or random blocks) should be used to minimise residual variations of environment or stress conditions. It is very difficult to control with large precision the level of soil hypoxia imposed to the different individuals, as the oxygen concentration in the soil depends also on rooting density, soil heterogeneity and the presence of soil microorganisms. One possibility to control more directly the amount of oxygen available to the root system is the use of hydroponic systems that are bubbled with a specific nitrogen/oxygen mixture (e.g. Ricard et al. 1998). However root growth; root anatomy and root system architecture differ widely between hydroponics and soils.

Some degree of intra-specific genetic diversity of traits induced by root hypoxia has been shown for a number of species and traits. In the first section of this chapter, we review common garden comparisons of natural populations which are exposed to different levels of waterlogging at their sites of origin. The second section concentrates on offspring from controlled crosses of specific genotypes, often preclassified as tolerant or sensitive to waterlogging, used for quantitative analyses of the genetic determinism, and ultimately for the detection of Quantitative Trait Loci (QTL).

12.2 Diversity Among Populations: Adaptation to Water-Logged Soils?

Common garden comparisons of individuals grown from seeds collected in diverse populations were mainly published for noncrop species. The detected diversity was interpreted, with some caution, as revealing differences in adaptation due to natural selection.

Interest in intra-species variation of tolerance to waterlogging or to flooding emerged in the 1970s. Some examples include among-family variation in *Veronica peregrina*, an annual dicotyledon found on moist sites (Linhart and Baker 1973); population differences in *Eucalyptus viminalis*, from dry or wet forests (Ladiges and Kelso 1977) or in *E. ovata,* Australian swamp gum (Clucas and Ladiges 1979). These studies showed already that phenoypic differences could be detected among populations from sites differing in susceptibility to waterlogging and hypoxia.

Growth is strongly affected by waterlogging, and is usually significantly depressed in plants from both well-drained and hydromorphic soils. This was the case for *Eucalyptus globulus* and *E. grandis* families (Marcar et al. 2002), where at least *E. globulus* is know to be sensitive to waterlogging. Similarly, during a hypoxia experiment on *Geum rivale* (wetland species)*, Geum urbanum* (dry habitat species) and hybrid populations, root dry weight was reduced in all families (Waldren et al. 1988). However, as examples span a large range of plant species, from monocotyledon grasses to forest trees, responses are very diverse. In some species, growth increased during waterlogging for populations from wet environments, such as in *Panicum antidotale* (Ashraf 2003) or *Paspalum dilatatum* (Loreti and Oesterheld 1996) populations, whereas it decreased in other species. This differential growth response to waterlogging is an extreme case of environment and genotype interaction and can also be found to a lesser degree within species level. Marcar et al. (2002) studied growth under waterlogging in different populations from two *Eucalyptus* species. A significant treatment and provenance effect was detected for shoot dry weight in *E. globulus*, a rather hypoxia-sensitive species, but none for *E. grandis*, a species growing on hydromorphic soils. Similarly, Waldren et al. (1988) found in *G. rivale* no population differentiation for growth during waterlogging, whereas *G. urbanum* and hybrid populations showed significant population and waterlogging interactions. Overall, these examples show that a diversity of adaptations to waterlogging can evolve in closely related species resulting in growth differences. This suggests a genetic differentiation among populations, and thus a genetic determinism of hypoxia tolerance. However, there are also examples of species where no specific adaptation has been detected for hypoxia-exposed populations that would result in growth differences. In *Acer rubrum* seedlings, no relationship was detected between population differences in response to controlled waterlogging and the maternal hydrologic conditions (Will et al. 1995).

Adaptive differences among populations have also been detected using net CO_2 assimilation rate and stomatal conductance as indicators of fitness on lowland (wet) and upland (dry) populations of *P. dilatatum* (Mollard et al. 2008). Flooded plants displayed higher net CO_2 assimilation and stomatal conductance compared to controls in lowland populations, and stomatal closure and reduced net CO_2 assimilation in upland populations: under similar hypoxia, lowland populations were able to maintain water absorption by roots, while upland populations were not.

Variability in growth and leaf gas exchange among genotypes during waterlogging is the result of anatomical or physiological adaptations, such as the ability to develop hypertrophied lenticels, adventitious roots and aerenchyma in root or stem tissue. Under waterlogging, a significant increase in the number and height of hypertrophied lenticels was found in populations of *Luehea divaricata* from temporarily waterlogged soils versus those from well drained soils (De Carvalho et al. 2008, C.F. Ruas, pers. comm.). The development of hypertrophied lenticels or adventitious roots is typically an induced adaptive response to hypoxia with quite a large genetic diversity. A genetic basis for diversity in adventitious root growth was detected among *Carex flacca* populations (Heathcote et al. 1987). Continuous flooding increased adventitious root biomass in all populations to the same extent. Significant population differences and population and treatment interactions were detected during repeated transient episodes of flooding. This underlines that the modality of stress application may impact the degree of genetic diversity detected. Stress responses also often differ between organs, as in *P. dilatatum*, where porosity did not increase in roots during flooding, while it did in the leaf sheath (Mollard et al. 2008). However, there were strong treatments and population interactions: root porosity was different between lowland (wet) and upland (dry) populations under control conditions but not under flooding, whereas leaf sheath porosity was different under flooding but not under control conditions. The hypoxia-adapted lowland populations had constitutively higher root porosity, with little increase during flooding, whereas upland populations showed a larger response to flooding for leaf sheath porosity. Overall, these examples, covering a range of different plant types, suggest that genetic differences seem to have evolved for morphological adaptations to root hypoxia among natural populations exposed to different levels of soil hypoxia and that an adaptation to different environments has taken place.

As described above, hypoxia induces changes in root metabolism. Genetic differences in the expression of alcohol dehydrogenase (ADH) have been studied intensively in a number of species. As early as the 1970s, ADH polymorphism has been shown to affect growth rate under waterlogging (Marshall et al. 1973; Brown et al. 1976) and population differences were detected (Torres et al. 1977; Brown 1978). A genetic variability was also detected for the gene coding of ADH (locus ADH-B) among five European populations of *Fraxinus excelsior,* but was not related to flooding frequency at the sites of origin (Ruedinger et al. 2008). Herzog and Krabel (1999) studied 17 isoenzyme loci, of which some are thought to be involved in waterlogging or hypoxia tolerance. They found no evidence for a selection on these loci when comparing a frequently flooded and a dry-land population of *Quercus robur*. Chan and Burton (1992) found for *Trifolium repens* a strong population and treatment interaction for ADH activity in roots, with higher activities in populations from frequently flooded sites. ADH activity under waterlogging was positively correlated with relative growth rate, suggesting that a higher ADH activity contributes to a higher tolerance to hypoxia. This contradicts inter-specific comparisons, where more tolerant species displayed lower ADH activity (McManmon and Crawford 1971). However, ADH activity varies with time during stress application: sensitive *Brassica rapa* L. plants displayed a higher ADH activity after 18 h of stress but not earlier or later (Daugherty and Musgrave 1994). Enzymes potentially involved in hypoxia tolerance have been studied in detail in diverse crop species, where genetically well-defined varieties or clones are available. Increased ADH activity was found for waterlogging tolerant compared to susceptible *Zea mays* genotypes (Zaidi et al. 2003). Similarly, more tolerant *Oryza sativa* cultivars with a higher internode elongation rate under hypoxic conditions (escape strategy) showed also higher ADH and pyruvate decarboxylase (PDC) activities and ATP concentration (Kato-Noguchi and Morokuma 2007). This difference in ADH activity seems specific for roots (Kato-Noguchi et al. 2003). Fukao et al. (2003) found with seeds of the weed *Echinochloa crus-galli* germinating under anoxic conditions, that aldolase, aldehyde dehydrogenase and PDC were more strongly induced in a tolerant compared to an intolerant variety, whereas sucrose synthase, enolase and ADH showed similar induction patterns for both. The occurrence of some genetic variation has been detected in the sequence, expression and activity of ADH, whereas less information is available for other enzymes involved in hypoxia responses. However, even for ADH, we still lack experimental support demonstrating that the genetic diversity that is observed results in variation in adaptation to waterlogging by natural populations. Such a demonstration could be provided, for example, by population genetic studies linking single nucleotide polymorphisms (SNP) within the ADH-gene or its promoter to survival and fitness in stressed environments. Further, theoretical population genetic models could then be applied (e.g., Beaumont and Nichols 1996), testing whether nucleotide differentiation patterns of SNP within the ADH-gene could depart from neutral patterns and result from natural selection. This has been done, for example, with candidate genes for drought tolerance in *Pinus pinaster* populations (Eveno et al. 2008).

12.3 Genetic Control of Traits Related to Hypoxia Tolerance

A more direct approach to estimate genetic control of traits related to hypoxia tolerance is the estimation of heritability, which is, in the simplest case, the ratio between genetic and total variance within a given experimental set up (Lynch and Walsh 1997). The calculation of the genetic variance requires not only controlled conditions for trait estimations, but also an assessment of the relatedness of individuals within the experimental set up, such as multi-parental crossings (diallels, half-dialleles, clonal repetitions, etc). This approach is rarely possible with wild populations, however, it has been frequently used for crops. Heritability is difficult to compare among experiments, as it depends on environmental variance induced by the specific experimental set up. However, it provides an indication of the importance of the genetic control on a trait in a given experiment and can be used to predict results of artificial and natural selection (Hartl and Clark 1997), where narrow-sense heritability (ratio of additive genetic variance to total variance) is more important for population responses to individual selection than broad sense heritability (ratio of total genetic variance to total variance).

Significant levels of heritability have been detected under waterlogging or flooding in a number of species for biomass and yield (Collaku and Harrison 2005, *Triticum aestivum;* Silva et al. 2007, *Z. mays*) as well as for traits assessing the sensitivity to hypoxia (e.g., the percentage of yellow leaf (Zhou et al. 2007b, *Hordeum vulgare*). However, without an estimate of heritability under controlled conditions, it is impossible to infer whether the observed genetic control refers to a constitutive or an induced trait. Marcar et al. (2002) compared *E. globulus* (hypoxia sensitive) and *E. grandis* (tolerant) seedlings for shoot dry weight changes under water logging relative to control conditions. Narrow sense heritability for this trait was higher in the tolerant species and lower in the sensitive one. This species x environment interaction, suggests a larger genetic control of growth during hypoxia for the adapted plants. Kolodynska and Pigliucci (2003) observed during a three-generation selection experiment with *Arabidopsis thaliana* that heritability changed in response to selection, and that morphological traits displayed increasing heritabilities compared to life-history traits. Selection did not alter the overall shape of reaction norms but lowered the phenotypic means of some traits. Hybrid families of *G. rivale* × *urbanum* (wetland x dryland species) showed no significant heritability for the response of shoot biomass to waterlogging (Waldren et al. 1988). However, the response of root dry weight or shoot/root ratio was under significant genetic control in this experiment. Thus, integrative traits with no direct link to hypoxia tolerance such as above-ground biomass yield or growth might, in some situations seem not to be under genetic control even though some genetic diversity was detected in adaptive traits. In such cases, differences in fitness during stress might be explained better by survival rate than by aboveground growth and biomass production.

A typical adaptive trait studied in rice is internode elongation during submergence. Nemoto et al. (2004) studied the lowest elongated internode (LEI) for a diallel crossing of different *Oryza sativa* and *O. rufipogon* varieties. For this highly adaptive trait, high heritabilities were estimated (0.994 for broad sense and 0.962 for narrow sense) with a much larger additive than dominant variance, suggesting a high potential for individual selection. The heritability of adventitious root development was studied under flooding in *Cucumis sativus* (Yeboah et al. 2008). Narrow-sense heritability was higher for this trait (0.74) than for the overall tolerance score (0.60). The heritability of total root dry weight was higher under waterlogging than in controls. In a large test with 436 *Z. mays* inbred lines, a low broad-sense heritability was found for root porosity under normal conditions, which increased significantly during waterlogging (Zaidi et al. 2007). In contrast, heritability of biomass and yield declined during waterlogging compared to control. The tight correlation between root porosity and grain yield under stress, and its absence in controls, stresses the importance of root porosity for hypoxia tolerance in this species. We found no estimate of heritability for the development of hypertrophied lenticels in the literature. Nevertheless, in general, whenever heritability was estimated for morphological adaptive traits, a rather tight genetic control was shown, and it often increased under stress. There are few estimates of genetic control of enzyme activities related to hypoxia tolerance. Chan and Burton (1992) showed a strong genetic control for hypoxia-induced ADH activity in *T. repens* populations (broad sense heritability 0.55±0.13). Overall, tolerance traits seem to show higher heritabilities in stressed conditions compared to productivity or traits quantifying damage or overall performance.

12.4 Genetic Determinism of Tolerance to Waterlogging and Identification of the Involved Genome Regions

Once the occurrence of a genetic control of a trait has been established, the next step is to identify the underlying genetic determinism, that is, how many genes control the expression of the trait and to what extent each gene controls its variability. The classical approach to this question is QTL (quantitative trait loci) mapping, the resolution of quantitative traits into discrete mendelian inherited components (Paterson et al. 1988). This requires a reference population screened for a high number ($\gg$100) of genetic markers. The recombination information produced by the progeny is then used to order the markers on a genetic map. The comparison of this genetic information across all individuals with their phenotype for a given trait allows identifying regions on the genetic map (QTL), that each determines a fraction of the observed phenotypic variability of the trait (called the phenotypically explained variance, PEV). The least likelihood (LOD), position on the genetic map, allelic substitution effect and PEV are estimated for each QTL. Depending on the statistical package used, the presence of a QTL is either determined by a LOD score threshold or a significance statistic calculated using permutation techniques. Further, bootstrap methods allow estimating standard deviations of all parameters.

However, most parameters estimated during QTL analyses, including the number of QTL detected, depend heavily on the number of genotypes in the reference population. Simulations showed that PEV values are overestimated and that the number of QTL detected does not correspond to the number of loci involved when the sample size of the mapping pedigree (N) is below 1,000 (Beavis 1994). QTL experiments for hypoxia-related traits never involved more that 300 genotypes (Table 12.2, range: 60–288), related to the large experimental set up, as for example, shown in Fig 12.1. Some caution is therefore needed when attempting to infer the actual genetic determinism of specific traits as the number of detected QTL is likely to be smaller than the actual number of genes involved. QTL experiments with a relatively small number of genotypes will mainly detect major QTL with high allelic substitution effects and PEV.

QTL detection encompassed the whole range of traits and conditions described above, focusing on short-term responses of physiological processes, or long-term acclimation with the objective to investigate morphological adaptations. The environmental conditions used for QTL detection, ranged from waterlogging and flooding to total submergence.

12.4.1 Methodology of the Detection of QTL for Hypoxia Tolerance: Caution and Strategies

12.4.1.1 Submergence Tolerance and Waterlogging Tolerance

To date, QTL detection for tolerance to total submergence concentrated on rice (Xu and Mackill 1996; Nandi et al. 1997; Sripongpangkul et al. 2000; Xu et al. 2000; Toojinda et al. 2003; GuangHeng et al. 2006; Hattori et al. 2007). All these experiments detected a major QTL on chromosome 9 (see Chen et al. 2002, for a detailed physical and genetic map of rice) and allowed the identification of the *sub-1* locus (Xu et al. 2006). QTL have also been detected for several species during partial submergence and root hypoxia (Vantoai et al. 2001; Zheng et al. 2003; Cornelious et al. 2005; Mano et al. 2005a, b; Cornelious et al. 2006; Githiri et al. 2006; Qiu et al. 2007; Parelle et al. 2007; Zhou et al. 2007a). Duration of waterlogging as well as the height of the water table were highly variable, ranging from a few days (Qiu et al. 2007) to several weeks (Vantoai et al. 2001; Mano et al. 2005b). and from few centimetres (Mano et al. 2005b; Qiu et al. 2007) to 10 cm above soil surface (Vantoai et al. 2001; Cornelious et al. 2005). This diversity in experimental procedures may have contributed to the large variability in the number and localization of the detected QTL (Table 12.2). Phenotyping after variable stress durations and intensities may detect different tolerance processes and thus result in a QTL detection that varies with environment. QTL detection is a statistical process, whereby minor QTL with low allelic substitution effects and thus low PEV will often be below the detection or significance limit. Parelle et al. (2007) detected a

Table 12.2 Number of QTL (N_Q) detected under hypoxic conditions (except Mano et al. 2007 and Mano et al. 2008) for different tolerance or growth traits; for each experiment (where n_i is the number of genotypes of the tested family)

Article	Species	n_i	Trait	N_Q	LOD score	PEV
Martin et al. (2006)	Iris	120	Survival	2	–	0.11–0.14
Qiu et al. (2007)	Maize	288	Total dry weight	4	2.8–5.9	0.12–0.32
Qiu et al. (2007)	Maize	288	Shoot dry weight	4	2.6–7.0	0.05–0.21
Qiu et al. (2007)	Maize	288	Root length	7	2.5–3.5	0.04–0.07
Qiu et al. (2007)	Maize	288	Root dry weight	2	2.7–2.9	0.04–0.05
Qiu et al. (2007)	Maize	288	Plant height	3	2.7–3.2	0.05–0.07
Mano et al. (2008)	Maize	195	Aerenchyma	5	1.5–3.4	0.04–0.09
Mano et al. (2007)	Maize	141	Aerenchyma	6	1.7–4.9	0.07
Mano et al. (2006)	Maize	178	Degree of leaf injury	1	4.4	0.03–0.15
Mano et al. (2005a)	Maize	201	Adventitious root formation	2	3.9–5.1	0.09–0.1
Mano et al. (2005a)	Maize	94	Adventitious root formation	1	6.5	0.25
Mano et al. (2005b)	Maize	110	Adventitious root formation	3	3.2–5.1	0.1–0.21
Parelle et al. (2007)	Oak	119	Hypertrophied lenticel Nr.	1	3.4	0.15
Parelle et al. (2007)	Oak	100	Epinasty	5	7.2–15.2	0.08–0.12
Parelle et al. (2007)	Oak	100	Hypertrophied lenticel dev.	1	2.8	0.1
Zhou et al. (2007a)	Rice	282	Seedling height	1	2.3	0.04
Zhou et al. (2007a)	Rice	282	Seedling emergence	2	2.1–3.9	0.04–0.07
Zhou et al. (2007a)	Rice	282	Coleoptile emergence	2	2.3–4.9	0.04–0.08
Zheng et al. (2006)	Rice	96	Seminal root length	1	2.6	0.12
Zheng et al. (2006)	Rice	96	Total root dry weight	3	3.0–3.5	0.13–0.16
Zheng et al. (2006)	Rice	96	Adventitious root number	2	0.53–2.98	0?–0.133
Zheng et al. (2003)	Rice	96	Seminal root length	1	2.6	0.12
Zheng et al. (2003)	Rice	96	Lateral root number	1	2.8	0.13
Zheng et al. (2003)	Rice	96	Lateral root length	2	2.4	0.12
Zheng et al. (2003)	Rice	96	Adventitious root number	4	2.5–4.6	0.11–0.20
Xu and Mackill (1996)	Rice	169	Tolerance	1	36	0.69
Toojinda et al. (2003)	Rice	172	Total shoot elongation	4	3.9–28.1	0.01–0.52
Toojinda et al. (2003)	Rice	65	Total shoot elongation	3	6.2–27.3	0.24–0.74
Toojinda et al. (2003)	Rice	172	Tolerance scrore	9	3.2–52.6	0.07–0.63
Toojinda et al. (2003)	Rice	65	Tolerance scrore	2	12.3–49.1	0.28–0.72

Toojinda et al. (2003)	Rice	172	Relative shoot elongation	2	3.8–16.2	0.01–0.12
Toojinda et al. (2003)	Rice	172	Per cent plant survival	4	5.4–65.8	0.11–0.77
Toojinda et al. (2003)	Rice	65	Per cent plant survival	3	5.4–17.3	0.16–0.48
Toojinda et al. (2003)	Rice	172	Leaf senescence	4	4.0–29.3	0.1–0.53
Toojinda et al. (2003)	Rice	65	Leaf senescence	2	5.5–18.0	0.30–0.72
Tang et al. (2005)	Rice	192	Early elongation ability	2	3.8–18.2	0.09–0.41
Sripongpangkul et al. (2000)	Rice	165	Survival (qualitative)	13	–	–
Sripongpangkul et al. (2000)	Rice	165	Plant height increment	11	3.6–13.0	0.05–0.26
Sripongpangkul et al. (2000)	Rice	165	Leaf increment	4	2.5–10.8	0.09–0.25
Sripongpangkul et al. (2000)	Rice	165	Initial plant height	9	4.0–10.1	0.06–0.31
Sripongpangkul et al. (2000)	Rice	165	Total increment	5	4.0–10.8	0.06–0.36
Sripongpangkul et al. (2000)	Rice	165	Internode increment	3	7	0.09–0.37
Nemoto et al. (2004)	Rice	186	Early elongation ability	1	18.6	0.46
Nandi et al. (1997)	Rice	74	Survival (qualitative)	1	–	–
Nandi et al. (1997)	Rice	250	Survival	4	3.2–4.7	0.19–0.27
Ikeda et al. (2007)	Rice	98	No QTL specific for treatment			
Hattori et al. (2007)	Rice	94	Total internode elongation	5	3.4–6.2	0.17–0.36
Hattori et al. (2007)	Rice	94	Elongated internode Nr.	2	4.5–6.2	0.27
Hattori et al. (2007)	Rice	94	Lowest elongation internode	3	3.1–7.7	0.14–0.36
GuangHeng et al. (2006)	Rice	–	Relative damage	3	2.8–4.6	–
GuangHeng et al. (2006)	Rice	–	Plant height	3	2.8–3.9	–
GuangHeng et al. (2006)	Rice	–	Livabilitysurvival	3	3.0–3.7	–
GuangHeng et al. (2006)	Rice	–	Lenghth of mesocotyl	4	2.3–3.8	–
GuangHeng et al. (2006)	Rice	–	Chlorophyll damage index	3	2.5–4.2	–
Vantoai et al. (2001)	Soybean	75	Seed yield	1	–	–
Vantoai et al. (2001)	Soybean	102	Seed yield	1	–	–
Vantoai et al. (2001)	Soybean	75	Growth	1	–	–
Vantoai et al. (2001)	Soybean	102	Growth	1	–	–
Githiri et al. (2006)	Soybean	60	Tolerance index	7	2.0–15.4	0.07–0.49
Cornelious et al. (2005)	Soybean	67	Tolerance	1	2	0.16
Cornelious et al. (2005)	Soybean	103	Tolerance	1	2.5	0.06
Finch-Savage et al. (2005)	Wild Mustard	95	Germination	1	2.8	0.13

Fig. 12.1 Example of the experimental procedure for QTL detection of waterlogging tolerance traits (Parelle et al. 2007). This photography shows 320 rooted cuttings of *Quercus robur* waterlogged for 4 weeks and phenotyped daily (photo Parelle)

QTL for epinasty of which the PEV varied from 2.8% to 11.6% depending on the observation date during permanent waterlogging. Furthermore, as discussed above, the estimation of PEV and allelic substitution effect of QTL also depends on the number of genotypes and of vegetative copies within genotypes. This, together with the variations in environmental conditions, makes it difficult to compare QTL among different experiments. However, comparing the position of major QTL across experiments clarifies their significance across conditions and genotypes.

One major QTL detected in rice during total submergence is the the *sub-1* locus (Xu and Mackill 1996), showing five to seven times higher LOD scores and at least two times higher PEV (Table 12.2) than QTL for any other adaptive trait despite the similar (±20%) number of genotypes involved in the different experiments, e.g.: Mano et al. (2007, 2008) and Mano and Omori (2008) for aerenchyma; Mano et al. (2005a, b) for adventitious roots; Mano et al. (2006) for leaf injury). Even when taking into account that the estimated LOD and PEV depend on the experiment, the *sub-1* locus stands out among all QTL detected for hypoxia tolerance. This might be due to the fact that it controls internode growth, which is a trait controlled probably by only few genes; whereas strategies of tolerance to waterlogging are more complex, probably relying on multiple traits, and thus depending on many, interacting genes. This would result in the detection of more QTL with lower PEV. However, other than indicating effectively a stronger genetic determinism, the high LOD scores for *sub-1* might also be due to the experimental control of the stress intensity. The high water table present during total submergence experiments homogenizes the hypoxic stress among plants, thus reducing within-experiment environmental variability; whereas hypoxic stress during waterlogging experiments also depends on the homogeneity of the soil and the rooting density. As oxygen diffusion in water is slow, the actual oxygen deficiency in the soil depends on root and rhizospheric O_2 consumption, which creates a gradient from the soil surface to the root. This induces a large variability of the stress actually perceived among plants within an experiment, with evident effects on the phenotypes and on QTL detection. Indeed Parelle et al. (2007) detected QTL for dissolved oxygen content in water in the vicinity of the roots of oaks submitted to waterlogging, revealing a clear problem of stress control among plants, with genotypes influencing their environment.

12.4.1.2 QTL Detection for Constitutive Traits of Tolerance

The most recently published experiments on maize (Mano et al. 2007, 2008) detected QTL for aerenchyma formation, a key trait known to be highly related to hypoxia tolerance. This was achieved with an inter-specific cross between two species with different capacities of aerenchyma formation. They performed the analysis only under control conditions, which avoids stress heterogeneity among individuals (although not environmental variability). Aerenchyma formation is usually enhanced under stress, thereby showing genotype x environment interactions and a different genetic determinism than under control conditions. This could change allelic effects and PEV of the detected QTL. Genotype x environment interaction could also be a cause for the small number of co-localised QTL between the two different inter-specific crosses in the same experimental set-up: only one out of seven detected QTL co-localized.

12.4.1.3 Comparison with a Control Environment

QTL detection for induced adaptive traits does not necessarily require a control treatment. Nevertheless, QTL detection for growth and productivity needs a comparison between stress and control. This is necessary to distinguish constitutive QTL that influence growth independently of the applied stress, and induced QTL that control growth specifically in response to stress. Qiu et al. (2007) and Githiri et al. (2006) compared the phenotypes expressed by the same genotypes growing in water-logged versus control conditions. Githiri et al. (2006) computed the ratio of seed production under waterlogging versus control, and used this ratio as a tolerance index. However, QTL for ratios between stress and control are difficult to interpret. This is mainly due to the fact that the condition that results in the highest among-genotype variability dominates the statistical analysis. Three situations might occur: (1) the variability is larger under stress, the QTL is then related to stress tolerance; (2) it is larger in controls (e.g. due to severe growth reduction under stress), and the QTL then describes the genetic variability of growth potential in the absence of stress, and not of tolerance; (3) the variability is similar in the two treatments and it is difficult to conclude whether the detected QTL is related to tolerance or not. Detecting QTL for each condition separately can provide support for the interpretation. This was for example used by Qiu et al. (2007), who detected QTL for the ratio of growth parameters between waterlogging and control as well as for each treatment separately and used the resulting co-localisations to interpret QTL as either nonadaptive, constitutive or induced. Other than using stressed/ control ratios as traits, both datasets can be used within the same statistical analysis, such as in multi-environment QTL detection models (Jansen et al. 1995) which allow a direct computation of QTL x environment interactions. This has been used for example by Jermstad et al. (2003) to detect QTL in a factorial experimental design using different winter chilling and spring flushing temperatures. Only one application of multi-environment QTL detection is known to the authors in the case of waterlogging. Parelle et al. (2007) recorded epinasty, root collar diameter and leaf chlorophyll content in a Q. *robur* full-sib progeny and showed that the allelic substitution effects of the detected QTL varied significantly during the 4 weeks of waterlogging. This method described QTL by the temporal pattern of the corresponding allelic substitution effect, and compared such patterns among different QTL and traits. Interestingly QTL with correlated effect patterns were dispersed over the whole genome, suggesting a polygenic determinism of tolerance to hypoxia.

12.4.2 Major Loci Detected for Hypoxia Tolerance

One of the main tools for breeding crops for agriculture in areas submitted to waterlogging or submergence is the detection of genomic regions or genes for marker aided selection (Vartapetian 2005). On the other hand, QTL studies also aim at elucidating the molecular mechanisms of hypoxia tolerance. However, only few QTL experiments were performed with the aim to detect loci for which the genetic diversity is effectively selected in natural populations. Table 12.1 summarizes the traits and species for which QTL related to hypoxia response were detected.

12.4.2.1 *QTL* for Traits Submitted to Natural Selection Pressure in Hypoxic Environments

Identifying candidate genes submitted to natural selection in water-logged or flooded soils would advance our understanding of speciation processes in such environments (Lexer et al. 2005). Martin et al. (2006) detected two QTL for survival in an Iris family. Their experiment was performed under water-logged conditions in a common garden for 4 years. Another experiment was performed by Parelle et al. (2007) who detected QTL for traits that are known to vary among natural population of two sympatric, hybridising oak species (*Q. robur* and *Q. petraea*). They detected two QTL for hypertrophied lenticel formation, five for the level of epinasty, but none for adventitious root development. Loci identified during these experiments could be a starting point in research strategies identifying candidate genes. Such genes could then be screened for genetic variability in natural populations.

12.4.2.2 QTL Detection for Breeding Purposes

Many QTL were detected for a large range of traits of interest for the maintenance of productivity and growth under waterlogging (see Table 12.2 for details). Shoot growth was the main indicator of productivity during hypoxia, and QTL were detected for shoot biomass by Qiu et al. (2007) or shoot height by GuangHeng et al. (2006) and Qiu et al. (2007). Vantoai et al. (2001) used shoot growth during stress as a tolerance index, considering that eliminating the growth before stress would detect induced rather than constitutive QTL. As the root system is directly affected by hypoxia, some authors use it as an indicator of tolerance, for example Qiu et al. (2007) detected QTL for root length under hypoxic condition, or Zheng et al. (2006) for total root biomass. QTL detected for these traits might be used for marker aided selection or to produce inbred lines to improve crop performance under hypoxia (Vantoai et al. 2001), without necessarily having detailed information on the tolerance strategy that is controlled by the QTL.

12.4.2.3 QTL Detection for Tolerance to Hypoxia

The most recent studies on QTL detection of hypoxia tolerance were performed on hypoxia-induced morphological traits: 11 QTL were identified for aerenchyma formation in maize (Mano et al. 2007, 2008) and two QTL for hypertrophied lenticel formation in oaks (Parelle et al. 2007). Six QTL for adventitious root development were detected in maize (Mano et al. 2005a, b), but none in *Q. robur* despite a visible development of such roots. This lack of genetic determinism for adventitious root development in oak might be due to heterogeneity of soil hypoxia, which dominated the phenotypic variance of this trait, and therefore diluted the genetic variance (Parelle et al. 2007).

To characterise the tolerance of rice to total submergence, internode elongation was used to quantify the capacity of quiescence or stress escape. In consequence, this trait allowed the detection of two types of loci related to those two strategies. This can be used in QTL detection studies when specific crosses are used, either combining two genotypes with different strategies or a tolerant and a non tolerant genotype. Indeed, the sign of the allelic substitution effect, combined with the knowledge of the strategy developed by the parents allowed to clearly attribute

Table 12.3 Traits for which a QTL related to an allele of the F13 A variety of *Oryza sativa* ssp *indica* was detected at the Sub1 Loci

Trait	Article	Methods of detection	LOD score	R^2
Tolerance scrore (visual scale)	Xu and Mackill 1996	Linear regression	36	0.69
Surviving or not	Nandi et al. 1997	Direct mapping of the qualitative trait	–	–
Per cent plant survival	Toojinda et al. 2003	Composite interval mapping	36.4–65.8	0.41–0.77
Relative shoot elongation compare to control			16.2	9.7
Tolerance score (visual scale)			26.6–38.3–52.6	0.38–0.61–0.63
Leaf senescence after submergence			29.3	0.53

For the LOD score and the R^2 values among repetitions of the QTL detection experiments are indicated

QTL to the two strategies. Several authors (Sripongpangkul et al. 2000; Toojinda et al. 2003; Nemoto et al. 2004; Tang et al. 2005; Hattori et al. 2007; Kawano et al. 2008) detected QTL related to the escape strategy of different rice varieties, and Xu and Mackill (1996) detected the *sub-1* locus related to the quiescence strategy by an inhibition of the internode elongation. In Table 12.3 all traits are listed for which QTL were detected at the *sub-1* locus. Sripongpangkul et al. (2000) and Hattori et al. (2007) performed multiple trait phenotyping of the early elongation ability, where the constitutive diversity included in each trait differed, whereas stress responses relating to the same tolerance mechanism would result in co-localisation of QTL. Both experiments resulted in the detection of the sub-1 locus, thus relating it clearly to low elongation ability (quiescence strategy). An important step for breeding was the successful introgression of the sub-1 locus into a rice variety of economic importance. Siangliw et al. (2003) crossed three tolerant varieties of rice, containing the *sub-1* allele conferring tolerance by quiescence strategy, with the hypoxia intolerant Thai jasmine rice. In the hybrid families, QTL were detected for hypoxia tolerance at the *sub-1* locus and the alleles related to quiescence were always from the tolerant parent. Introgression increased survival from 1.6% in Thai jasmine to 23%–31% in the hybrid families. This example shows how the detection of a QTL for tolerance can be used directly for breeding purposes.

The *sub-1* locus was the only QTL for which the underlying genes were clearly identified. It was reported for the first time by Xu and Mackill (1995, 1996), and further detected during all QTL detection experiments in rice, including the tolerant variety F13A, in which elongation is inhibited in order to decrease energy demand during hypoxia (quiescence strategy) (Nandi et al. 1997; Toojinda et al. 2003). Nandi et al. (1997) demonstrated that a, cartography of the qualitative trait “surviving/not surviving to total submergence” was sufficient to detect the *sub-1* locus.

The *sub-1* locus was not only detected in F13A, but also in other tolerant varieties. For example Sripongpangkul et al. (2000) detected a QTL for elongation ability on the *sub-1* locus in a F8 RIL cross from two *indica* cultivars; the tolerant parent conferring the allele for a, faster elongation ability. This locus therefore seems to be also involved in the escape strategy developed by some deep-water varieties of rice. However, the main QTL for the fast elongation capacity was detected on a different chromosome than *sub-1* (Tang et al. 2005; Hattori et al. 2007, 2008).

Candidate gene approaches were used to identify the gene(s) beneath the *sub-1* locus. Ruanjaichon et al. (2004) first mapped a small GTP-binding protein, belonging to a family known to be involved in signal transduction pathways and Kottapalli et al. (2006) identified 1,473 (putative) genes. However the large confidence interval for the position of the QTL for *sub-1* (6,4 cM for Kottapalli et al. 2006) did not allow the identification of the gene(s) responsible for submergence tolerance. It was finally the sequencing of the rice genome (International Rice Genome Sequencing Project 2005), the construction of a high resolution genetic map (Harushima et al. 1998; Xu et al. 2000) and the comparison of genetic and physical maps (Kamolsukyunyong et al. 2001; Chen et al. 2002) that allowed the identification of the *sub-1* cluster of genes (Xu et al. 2006). To date, the physical structure of this locus is well known (see Fukao et al. 2006 and Xu et al. 2006 for details). This locus contains 13 genes, including three ethylene response factors called Sub1-A, Sub1-B and Sub1-C. Sub1-A is present only in *O. sativa* ssp *indica*, including the tolerant variety F13A (Xu et al. 2006; Fukao et al. 2009). This gene originates probably from the duplication of the Sub1-B gene, as the two genes display a large sequence homology, and as the presence of the Sub1-A gene was correlated with variation of Sub1-B alleles (Fukao et al. 2009). Two alleles of Sub1-A, have been reported: the Sub1-A-1 and the Sub1-A-2. These alleles were correlated with variation of alleles of Sub1-C (Fukao et al. 2009). The tolerant variety F13A possesses the Sub1-A-1 allele and the corresponding alleles of the Sub1-C and Sub1-B. Recombinant crossing experiments among the three genes (Xu et al. 2006; Septiningsih et al. 2009) demonstrated that variation of the two alleles of the Sub1-A locus modify the submergence tolerance of rice, independently of the effect of the alleles present in Sub1-B and Sub1-C. This suggests that the QTL is controlled by the allelic effect of one single gene, of which only two states were detected: presence / absence of the Sub1-A-1 allele or the presence / absence of the entire gene. Introgression of the Sub1-A-1 allele into other species than rice could improve productivity of crops under flooding. The Sub1-A-1 allele induces the quiescence strategy resulting from the inhibition of internode elongating (Toojinda et al. 2003). Actually the Sub1-A-1 allele inhibited the effects of the Sub1-C gene on elongation initiation in response to ethylene (Fukao et al. 2006). However, it is far from being clear whether the introgression of the Sub1-A-1 allele into other species actually confers a larger tolerance to total submergence, as interactions with other tolerance strategies need to be taken into account.

It is interesting to notice that the three *sub1* genes (A, B, and C) are ethylene response factors. Ethylene is known to be involved in a large number of hypoxia tolerance mechanisms, as for example the development of aerenchyma, adventitious roots and hypertrophied lenticels (Bailey-Serres and Chang 2005). To our knowledge no QTL experiment was performed directly for ethylene production or for other traits related to signalling of hypoxia stress.

12.5 Conclusions

Genetic diversity has been shown among populations or within mapping families, for indirect indicators of tolerance, such as growth and leaf level gas exchange, as well as for constitutive or induced adaptive traits. The genetic control was elucidated for only a small number of traits and even less gene candidates have actually been tested. At the enzyme level, some diversity was detected only for ADH. However, no signal transduction pathway has been put forward and related to the observed genetic differences. Further, the actual effect of genetic differences in ADH on survival and fitness in natural populations still lacks experimental support. The correlation between the observed genetic diversity of short term metabolic adjustments to hypoxia and long term morphological adaptations needs further investigation. The QTL detected for survival or traits known to vary among natural populations could be starting points for gene candidate approaches. Such gene candidates could then be screened for natural genetic variability, thereby generating knowledge on the adaptability of populations, especially with respect to environmental changes.

The major challenge for future QTL detection for traits conferring hypoxia tolerance is the definition of integrative traits (1) indicating different tolerance strategies and (2) well suited to high-throughput phenotyping required for quantitative genetic analyses. In addition to the *sub-1* locus, a large number of minor QTL have been detected. The combination of several favourable alleles will determine the tolerance of an individual. However to decompose these processes, future approaches should combine large scale QTL experiments using complex traits and detailed studies on selected genotypes to decompose overall tolerance into elementary components.

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