

# Genetic variability and determinism of adaptation of plants to soil waterlogging

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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 5 the growth and fitness of plant species, from crops to forest ecosystems. An 6 improved understanding of the intra-species genetic diversity of traits involved in 7 hypoxia tolerance is a prerequisite for crop breeding programmes aimed at increas- 8 ing the tolerance to waterlogging, as well as for assessing the adaptability of natural 9 populations to waterlogging. Some genotypes within the species have developed 10 adaptations to hypoxia, as shown by differences among populations in growth and 11 fitness, and in traits conferring some degree of tolerance such as sequence, expres- 12 sion and activity of alcohol dehydrogenase, or the ability to develop adventitious 13 roots, increased tissue porosity and hypertrophied lenticels. Genetic control has 14 been estimated for a number of such traits. Overall, under waterlogging, specific 15 tolerance traits show higher heritabilities compared to traits quantifying productiv- 16 ity, damage or overall performance. Genomic regions involved in the control of 17 these traits (i.e., Quantitative Trait Loci QTL) have been detected for tolerance 18 traits in a few species, and allow gaining some insight into the genetic basis of the 19 observed natural diversity or may be a starting point for breeding purposes. 20 However, only for submergence tolerance in rice (sub-1) has a successful gene 21 candidate approach resulted in the detection of alleles that are directly involved in 22 the tolerance process. 23

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### 24 Abbreviations

- 25 ADH Alcohol dehydrogenase
- 26 LEI Lowest elongated internode
- 27 PDC Pyruvate decarboxylase complex
- 28 PEV Per cent of explained variance
- 29 QTL quantitative trait loci
- 30 RIL Real isogenic lines
- 31 SNP Single nucleotide polymorphism
- 32 Sub Submergence tolerance locus

## 33 12.1 Introduction

Excess soil water due to flooding or temporary waterlogging can be a major 34 35 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 distri-36 bution of plant species in natural environments. Some species or genotypes within 37 the plant species have developed adaptive responses to flooding and waterlogging. 38 In the case of crops, the occurrence of some genetic diversity in tolerance traits is a 39 40 prerequisite for breeding programmes. In natural ecosystems, due to the local occurrence of temporarily waterlogged soils (often called hydromorphic soils, 41 Lévy et al. 1999), the frequency and severity of episodes of waterlogging or 42 flooding act as a selective pressure and differences in tolerance can develop 43 among species, or populations within species. To gain insight into the degree of 44 45 inter-specific variability, we need a careful quantification of the tolerance to waterlogging in individuals and methods to assess it as objectively as possible. 46

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 54 following the onset of waterlogging or flooding (Setter and Waters 2003). Due to 55 56 a reduced gas exchange between soil and atmosphere, changes in soil bacteria populations occur, oxygen concentration decreases rapidly (hypoxia), carbon diox-57 ide and ethylene concentrations increase, reduced and toxic cations such as manga-58 nese (Mn<sup>++</sup>) and iron (Fe<sup>++</sup>) accumulate, and an intense de-nitrification occurs. In 59 case of prolonged waterlogging, soils may be completely depleted of oxygen 60 61 (anoxia) and hydrogen sulphide and methane are produced and diffuse into the AU1

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atmosphere. Except the last, all of these steps occur usually within the first 20 days 62 of waterlogging. In some soils, this sequence may even occur faster. In this review, 63 we will concentrate on the hypoxia induced by waterlogging or total submergence 64 with all the consequences it might have on respiration, metabolism and growth of 65 affected plants. 66

To date, the processes conferring some degree of tolerance to waterlogging and 67 hypoxia are still not fully understood despite accumulating information (e.g., 68 Vartapetian 2006; Voesenek et al. 2006; Colmer and Voesenek 2009; Kawano 69 et al. 2009; Jackson et al. 2009; Parolin 2009). The degree of tolerance to a given 70 level of waterlogging may be assessed: (1) indirectly through damage indices or the 71 observed, usually negative, impact on growth, productivity and survival, or (2) 72 directly by evaluating the occurrence of traits contributing to the acclimation to 73 hypoxia (adaptive traits). These traits can be constitutive (i.e., they occur already in 74 individuals growing under optimal conditions and provide some advantage during 75 waterlogging) or induced (i.e., they appear only during episodes of waterlogging 76 in response to a signalling cascade). Induced traits can be roughly grouped into 77 short-term responses (e.g., metabolic adjustments) and long-term acclimations 78 (e.g., development of aerenchyma). A typical short-term response of roots is a 79 decrease of respiration and an increase of glycolytic flux and alcoholic fermenta- 80 tion (Drew 1997). Some key enzymes in this process are alcohol dehydrogenase 81 (McManmon and Crawford 1971; Chan and Burton 1992; Bailey-Serres and 82 Voesenek 2008), sucrose synthase or hexokinases (Germain et al. 1997; Ricard 83 et al. 1998). Long-term responses are mainly related to growth, either of existing or 84 of newly formed structures. In rice, where total submergence clearly poses a major 85 problem for productivity (Tuberosa and Salvi 2004), the elongation of internodes is 86 an important adaptive trait, resulting either in quiescence or an escape strategy 87 (Bailey-Serres and Voesenek 2008). The quiescence strategy consists in a lack of 88 elongation (Xu and Mackill 1996), whereas the escape strategy consists in an 89 enhanced growth rate that maintains the top of the shoot above the water level 90 (Fukao et al. 2006). Adaptive morphological traits are slower to develop compared 91 to purely physiological or metabolic adjustments. Assessment of such traits requires 92 long-term experiments with the risk of an interaction between ontogenic develop- 93 ment and stress response. A few anatomical traits, thought to allow transport of 94 oxygen to roots and enable a partial maintenance of respiration, survival or even 95 root growth, have commonly been measured in experiments on genetic variability. 96 They include the development of hypertrophied lenticels (Parelle et al. 2007), of 97 adventitious roots (Mano et al. 2005a, b) and of aerenchyma (porosity) in root or 98 stem tissues (Zaidi et al. 2007; Mollard et al. 2008; Mano et al. 2007, 2008; Mano 99 and Omori 2008; and see also Chap. 6 in this volume). 100

From an agronomic point of view, the maintenance of productivity, particularly 101 yield, is of major importance. This can be evaluated by quantifying growth or 102 biomass and also more indirectly by assessing, among others, leaf level gas 103 exchange or photosynthetic capacity. Leaf gas exchange, for instance, has been 104 used (Dreyer 1994; Wagner and Dreyer 1997) to characterise the overall 105 performance under waterlogging. Such traits bring no information about the 106

107 morphological and physiological mechanisms of tolerance; nevertheless, maintenance of productivity or photosynthesis contributes to fitness and survival of 108 individuals. Survival rate under hypoxia is, together with shoot dieback and other 109 fitness related traits (number of seeds produced, etc), an important means to assess 110 the degree of tolerance of populations. Leaf epinasty, the downward growth of leaf 111 petioles, is a specific response to root hypoxia in some species (Jackson and 112 Campbell 1976) and a direct indicator of the level of hypoxia stress perceived by 113 the individuals (Vartapetian and Jackson 1997). All these traits may respond to 114 waterlogging with quite different intensities. On the other hand, some traits obvi-115 ously play a direct adaptive role, or at least are thought to do so. Such traits include 116 the development of hypertrophied lenticels, of adventitious roots, of aerenchyma or 117 the occurrence of physiological changes (switch from a respiratory to a fermenta-118 tive metabolism). All these traits contribute to mitigate the impact of hypoxia in the 119 soil, by maintaining a minimal supply of oxygen to roots. 120

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

	different species			
t1.2	Stress type	Article	Genus	Trait types
t1.3	Submergence	Xu and Mackill (1996)	Rice	Damage
t1.4		Toojinda et al. (2003)	Rice	Damage
t1.5		Nandi et al. (1997)	Rice	Survival
t1.6		Sripongpangkul et al. (2000)	Rice	Survival
t1.7		Toojinda et al. (2003)	Rice	Survival
t1.8		Toojinda et al. (2003)	Rice	Growth
t1.9		Ikeda et al. (2007)	Rice	Growth
t1.10		Nemoto et al. (2004)	Rice	Elongation
t1.11		Tang et al. (2005)	Rice	Elongation
t1.12		Hattori et al. (2007)	Rice	Elongation
t1.13		Sripongpangkul et al. (2000)	Rice	Elongation
t1.14		Toojinda et al. (2003)	Rice	Elongation
t1.15	Waterlogging	Mano et al. (2006)	Maize	Damage
t1.16		Cornelious et al. (2005)	Soybean	Damage
t1.17		Martin et al. (2006)	Iris	Survival
t1.18		Vantoai et al. (2001)	Soybean	Growth
t1.19		Parelle et al. (2007)	Oak	Growth
t1.20		Qiu et al. (2007)	Maize	Growth
t1.21		Vantoai et al. (2001)	Soybean	Yield
t1.22		Githiri et al. (2006)	Soybean	Yield
t1.23		Mano et al. (2005a)	Maize	Adventitious roots
t1.24		Mano et al. (2005b)	Maize	Adventitious roots
t1.25		Zheng et al. (2003)	Rice	Adventitious roots
t1.26		Parelle et al. (2007)	Oak	Hypertrophied lenticels
t1.27	Control	Mano et al. (2007, 2008)	Maize	Aerenchyma

t1.1 **Table 12.1** Traits tested during QTL experiments to identify hypoxia-tolerance related loci in different species

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et al. 2006). However, the use of these traits to detect genetic differences in 126 waterlogging tolerance requires a careful interpretation to identify adaptive traits 127 involved in the tolerance to hypoxia, in contrast to nonadaptive traits indicating 128 merely a genetic difference, for instance in growth potential.

The quantification of damage induced by waterlogging may also provide an 130 estimation of tolerance. Some authors use visual ordinal scales of damage (Xu and 131 Mackill 1996; Sripongpangkul et al. 2000; Cornelious et al. 2005), others quantify 132 leaf senescence (Toojinda et al. 2003), fraction of yellow leaves (Zhou et al. 2007a) 133 or decline in leaf chlorophyll content (GuangHeng et al. 2006). Damage indices 134 have been successfully used to study the genetic determinism of tolerance in crops. 135 For example, the so-called *sub-1* locus (Xu and Mackill 1996) was identified in rice 136 10 years before the actual process controlled by the locus was understood (Xu et al. 137 2006). A large amount of damage eventually leads to mortality, which is a very 138 simple approach to characterising tolerance (Nandi et al. 1997; Sripongpangkul 139 et al. 2000; Toojinda et al. 2003; Martin et al. 2006). Despite the fact that survival is 140 an ordinal trait, the approximately normal distribution within experiments allows 141 using it for a genetic trait dissection in rice (Xu and Mackill 1996) and soybean 142 (Cornelious et al. 2005). The advantage of survival and damage traits is that the 143 variability tested is directly related to the stress tolerance, and can therefore be fully 144 attributed to genetic diversity. 145

To assess the genetic variability of tolerance to waterlogging or flooding, the 146 most obvious procedure is the quantitative analysis of traits conferring directly or 147 indirectly some level of tolerance to waterlogging. Prerequisites for suitable traits 148 include: (1) the relevance and specificity of the trait as an indicator of adaptation; 149 (2) the repeatability of the measurement procedure and (3) the possibility of 150 assessing a large number of individuals. 151

Genetic variability of tolerance traits can be studied in situ in natural popula- 152 tions only when detailed information on the environmental conditions and their 153 spatial and temporal variability is available. Further, an already advanced knowl-154 edge of the genetic determinism of the trait studied is necessary, with a remaining 155 risk of confusion between purely genetic differences and genotype and environ-156 ment interactions. Therefore, all of the studies reviewed here were done using 157 common conditions for all genotypes (vegetative copies or half-sib families), as 158 provided by common-garden plantations (comparative plantations with a homo-159 genized environment) or greenhouse experiments. However, even under such 160 controlled conditions, statistical methods (such as complete or random blocks) 161 should be used to minimise residual variations of environment or stress condi-162 tions. It is very difficult to control with large precision the level of soil hypoxia 163 imposed to the different individuals, as the oxygen concentration in the soil 164 depends also on rooting density, soil heterogeneity and the presence of soil 165 microorganisms. One possibility to control more directly the amount of oxygen 166 available to the root system is the use of hydroponic systems that are bubbled with 167 a specific nitrogen/oxygen mixture (e.g. Ricard et al. 1998). However root 168 growth; root anatomy and root system architecture differ widely between hydro-169 ponics and soils. 170

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).

# 179 12.2 Diversity Among Populations: Adaptation 180 to Water-Logged Soils?

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

Interest in intra-species variation of tolerance to waterlogging or to flooding 185 emerged in the 1970s. Some examples include among-family variation in Veron-186 ica peregrina, an annual dicotyledon found on moist sites (Linhart and Baker 187 1973); population differences in Eucalyptus viminalis, from dry or wet forests 188 (Ladiges and Kelso 1977) or in E. ovata, Australian swamp gum (Clucas and 189 Ladiges 1979). These studies showed already that phenoypic differences could be 190 detected among populations from sites differing in susceptibility to waterlogging 191 and hypoxia. 192

Growth is strongly affected by waterlogging, and is usually significantly depressed 193 in plants from both well-drained and hydromorphic soils. This was the case for 194 Eucalyptus globulus and E. grandis families (Marcar et al. 2002), where at least 195 E. globulus is know to be sensitive to waterlogging. Similarly, during a hypoxia 196 experiment on Geum rivale (wetland species), Geum urbanum (dry habitat species) 197 and hybrid populations, root dry weight was reduced in all families (Waldren et al. 198 1988). However, as examples span a large range of plant species, from monocoty-199 ledon grasses to forest trees, responses are very diverse. In some species, growth 200 increased during waterlogging for populations from wet environments, such as 201 in Panicum antidotale (Ashraf 2003) or Paspalum dilatatum (Loreti and Oesterheld 202 1996) populations, whereas it decreased in other species. This differential growth 203 204 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. 205 (2002) studied growth under waterlogging in different populations from two 206 *Eucalyptus* species. A significant treatment and provenance effect was detected 207 for shoot dry weight in E. globulus, a rather hypoxia-sensitive species, but none 208 for E. grandis, a species growing on hydromorphic soils. Similarly, Waldren et al. 209 210 (1988) found in G. rivale no population differentiation for growth during

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waterlogging, whereas *G. urbanum* and hybrid populations showed significant 211 population and waterlogging interactions. Overall, these examples show that a 212 diversity of adaptations to waterlogging can evolve in closely related species 213 resulting in growth differences. This suggests a genetic differentiation among 214 populations, and thus a genetic determinism of hypoxia tolerance. However, there 215 are also examples of species where no specific adaptation has been detected for 216 hypoxia-exposed populations that would result in growth differences. In *Acer* 217 *rubrum* seedlings, no relationship was detected between population differences in 218 response to controlled waterlogging and the maternal hydrologic conditions (Will 219 et al. 1995).

Adaptive differences among populations have also been detected using net 221  $CO_2$  assimilation rate and stomatal conductance as indicators of fitness on low-222 land (wet) and upland (dry) populations of *P. dilatatum* (Mollard et al. 2008). 223 Flooded plants displayed higher net  $CO_2$  assimilation and stomatal conductance 224 compared to controls in lowland populations, and stomatal closure and reduced 225 net  $CO_2$  assimilation in upland populations: under similar hypoxia, lowland 226 populations were able to maintain water absorption by roots, while upland populations were not. 228

Variability in growth and leaf gas exchange among genotypes during water-229 logging is the result of anatomical or physiological adaptations, such as the ability 230 to develop hypertrophied lenticels, adventitious roots and aerenchyma in root or 231 stem tissue. Under waterlogging, a significant increase in the number and height 232 of hypertrophied lenticels was found in populations of Luehea divaricata from 233 temporarily waterlogged soils versus those from well drained soils (De Carvalho 234 et al. 2008, C.F. Ruas, pers. comm.). The development of hypertrophied lenticels 235 or adventitious roots is typically an induced adaptive response to hypoxia with 236 quite a large genetic diversity. A genetic basis for diversity in adventitious root 237 growth was detected among *Carex flacca* populations (Heathcote et al. 1987). 238 Continuous flooding increased adventitious root biomass in all populations to the 239 same extent. Significant population differences and population and treatment 240 interactions were detected during repeated transient episodes of flooding. This 241 underlines that the modality of stress application may impact the degree of genetic 242 diversity detected. Stress responses also often differ between organs, as in 243 P. dilatatum, where porosity did not increase in roots during flooding, while it 244 did in the leaf sheath (Mollard et al. 2008). However, there were strong treatments 245 and population interactions: root porosity was different between lowland (wet) 246 and upland (dry) populations under control conditions but not under flooding, 247 whereas leaf sheath porosity was different under flooding but not under control 248 conditions. The hypoxia-adapted lowland populations had constitutively higher 249 root porosity, with little increase during flooding, whereas upland populations 250 showed a larger response to flooding for leaf sheath porosity. Overall, these 251 examples, covering a range of different plant types, suggest that genetic differ-252 ences seem to have evolved for morphological adaptations to root hypoxia among 253 natural populations exposed to different levels of soil hypoxia and that an adapta-254 tion to different environments has taken place. 255

As described above, hypoxia induces changes in root metabolism. Genetic 256 differences in the expression of alcohol dehydrogenase (ADH) have been studied 257 intensively in a number of species. As early as the 1970s, ADH polymorphism has 258 been shown to affect growth rate under waterlogging (Marshall et al. 1973; Brown 259 et al. 1976) and population differences were detected (Torres et al. 1977; Brown 260 1978). A genetic variability was also detected for the gene coding of ADH (locus 261 ADH-B) among five European populations of Fraxinus excelsior, but was not 262 related to flooding frequency at the sites of origin (Ruedinger et al. 2008). Herzog 263 and Krabel (1999) studied 17 isoenzyme loci, of which some are thought to be 264 involved in waterlogging or hypoxia tolerance. They found no evidence for a 265 selection on these loci when comparing a frequently flooded and a dry-land 266 population of Quercus robur. Chan and Burton (1992) found for Trifolium repens 267 a strong population and treatment interaction for ADH activity in roots, with 268 higher activities in populations from frequently flooded sites. ADH activity under 269 waterlogging was positively correlated with relative growth rate, suggesting that a 270 higher ADH activity contributes to a higher tolerance to hypoxia. This contradicts 271 inter-specific comparisons, where more tolerant species displayed lower ADH 272 273 activity (McManmon and Crawford 1971). However, ADH activity varies with 274 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 275 Musgrave 1994). Enzymes potentially involved in hypoxia tolerance have been 276 studied in detail in diverse crop species, where genetically well-defined varieties 277 or clones are available. Increased ADH activity was found for waterlogging 278 tolerant compared to susceptible Zea mays genotypes (Zaidi et al. 2003). Simi-279 larly, more tolerant Oryza sativa cultivars with a higher internode elongation 280 rate under hypoxic conditions (escape strategy) showed also higher ADH and 281 pyruvate decarboxylase (PDC) activities and ATP concentration (Kato-Noguchi 282 and Morokuma 2007). This difference in ADH activity seems specific for roots 283 (Kato-Noguchi et al. 2003). Fukao et al. (2003) found with seeds of the weed 284 Echinochloa crus-galli germinating under anoxic conditions, that aldolase, alde-285 hyde dehydrogenase and PDC were more strongly induced in a tolerant compared 286 to an intolerant variety, whereas sucrose synthase, enolase and ADH showed 287 similar induction patterns for both. The occurrence of some genetic variation 288 has been detected in the sequence, expression and activity of ADH, whereas less 289 information is available for other enzymes involved in hypoxia responses. How-290 ever, even for ADH, we still lack experimental support demonstrating that the 291 genetic diversity that is observed results in variation in adaptation to waterlogging 292 by natural populations. Such a demonstration could be provided, for example, 293 294 by population genetic studies linking single nucleotide polymorphisms (SNP) within the ADH-gene or its promoter to survival and fitness in stressed environ-295 ments. Further, theoretical population genetic models could then be applied (e.g., 296 Beaumont and Nichols 1996), testing whether nucleotide differentiation patterns 297 of SNP within the ADH-gene could depart from neutral patterns and result from 298 natural selection. This has been done, for example, with candidate genes for 299 300 drought tolerance in *Pinus pinaster* populations (Eveno et al. 2008).

### **12.3 Genetic Control of Traits Related to Hypoxia Tolerance** 301

A more direct approach to estimate genetic control of traits related to hypoxia 302 tolerance is the estimation of heritability, which is, in the simplest case, the ratio 303 between genetic and total variance within a given experimental set up (Lynch and 304 Walsh 1997). The calculation of the genetic variance requires not only controlled 305 conditions for trait estimations, but also an assessment of the relatedness of 306 individuals within the experimental set up, such as multi-parental crossings (dia-307 llels, half-dialleles, clonal repetitions, etc). This approach is rarely possible with 308 wild populations, however, it has been frequently used for crops. Heritability is 309 difficult to compare among experiments, as it depends on environmental variance 310 induced by the specific experimental set up. However, it provides an indication of 311 the importance of the genetic control on a trait in a given experiment and can be 312 used to predict results of artificial and natural selection (Hartl and Clark 1997), 313 where narrow-sense heritability (ratio of additive genetic variance to total variance) 314 is more important for population responses to individual selection than broad sense 315 heritability (ratio of total genetic variance to total variance). 316

Significant levels of heritability have been detected under waterlogging or 317 flooding in a number of species for biomass and yield (Collaku and Harrison 318 2005, Triticum aestivum; Silva et al. 2007, Z. mays) as well as for traits assessing 319 the sensitivity to hypoxia (e.g., the percentage of yellow leaf (Zhou et al. 2007b, 320 Hordeum vulgare). However, without an estimate of heritability under controlled 321 conditions, it is impossible to infer whether the observed genetic control refers to a 322 constitutive or an induced trait. Marcar et al. (2002) compared E. globulus (hypoxia 323 sensitive) and E. grandis (tolerant) seedlings for shoot dry weight changes under 324 water logging relative to control conditions. Narrow sense heritability for this trait 325 was higher in the tolerant species and lower in the sensitive one. This species x 326 environment interaction, suggests a larger genetic control of growth during hypoxia 327 for the adapted plants. Kolodynska and Pigliucci (2003) observed during a three-328 generation selection experiment with Arabidopsis thaliana that heritability changed 329 in response to selection, and that morphological traits displayed increasing herit-330 abilities compared to life-history traits. Selection did not alter the overall shape of 331 reaction norms but lowered the phenotypic means of some traits. Hybrid families of 332 G. rivale  $\times$  urbanum (wetland x dryland species) showed no significant heritability 333 for the response of shoot biomass to waterlogging (Waldren et al. 1988). However, 334 the response of root dry weight or shoot/root ratio was under significant genetic 335 control in this experiment. Thus, integrative traits with no direct link to hypoxia 336 tolerance such as above-ground biomass yield or growth might, in some situations 337 seem not to be under genetic control even though some genetic diversity was 338 detected in adaptive traits. In such cases, differences in fitness during stress might 339 be explained better by survival rate than by aboveground growth and biomass 340 production. 341

A typical adaptive trait studied in rice is internode elongation during submer- 342 gence. Nemoto et al. (2004) studied the lowest elongated internode (LEI) for a 343

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 0.962 345 for narrow sense) with a much larger additive than dominant variance, suggesting a 346 high potential for individual selection. The heritability of adventitious root devel-347 opment was studied under flooding in Cucumis sativus (Yeboah et al. 2008). 348 Narrow-sense heritability was higher for this trait (0.74) than for the overall 349 tolerance score (0.60). The heritability of total root dry weight was higher under 350 waterlogging than in controls. In a large test with 436 Z. mays inbred lines, a low 351 352 broad-sense heritability was found for root porosity under normal conditions, which increased significantly during waterlogging (Zaidi et al. 2007). In contrast, herita-353 bility of biomass and yield declined during waterlogging compared to control. The 354 tight correlation between root porosity and grain yield under stress, and its absence 355 in controls, stresses the importance of root porosity for hypoxia tolerance in this 356 species. We found no estimate of heritability for the development of hypertrophied 357 lenticels in the literature. Nevertheless, in general, whenever heritability was 358 estimated for morphological adaptive traits, a rather tight genetic control was 359 shown, and it often increased under stress. There are few estimates of genetic 360 control of enzyme activities related to hypoxia tolerance. Chan and Burton (1992) 361 showed a strong genetic control for hypoxia-induced ADH activity in T. repens 362 populations (broad sense heritability  $0.55\pm0.13$ ). Overall, tolerance traits seem to 363 show higher heritabilities in stressed conditions compared to productivity or traits 364 quantifying damage or overall performance. 365

# 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 368 is to identify the underlying genetic determinism, that is, how many genes control 369 the expression of the trait and to what extent each gene controls its variability. The 370 classical approach to this question is QTL (quantitative trait loci) mapping, the 371 resolution of quantitative traits into discrete mendelian inherited components 372 (Paterson et al. 1988). This requires a reference population screened for a high 373 number ( $\gg$ 100) of genetic markers. The recombination information produced by 374 the progeny is then used to order the markers on a genetic map. The comparison of 375 this genetic information across all individuals with their phenotype for a given trait 376 allows identifying regions on the genetic map (QTL), that each determines a 377 fraction of the observed phenotypic variability of the trait (called the phenotypically 378 explained variance, PEV). The least likelihood (LOD), position on the genetic map, 379 allelic substitution effect and PEV are estimated for each QTL. Depending on the 380 statistical package used, the presence of a QTL is either determined by a LOD score 381 threshold or a significance statistic calculated using permutation techniques. 382 Further, bootstrap methods allow estimating standard deviations of all parameters. 383

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However, most parameters estimated during QTL analyses, including the number 384 of QTL detected, depend heavily on the number of genotypes in the reference 385 population. Simulations showed that PEV values are overestimated and that the 386 number of QTL detected does not correspond to the number of loci involved when 387 the sample size of the mapping pedigree (N) is below 1,000 (Beavis 1994). QTL 388 experiments for hypoxia-related traits never involved more that 300 genotypes 389 (Table 12.2, range: 60-288), related to the large experimental set up, as for 390 example, shown in Fig 12.1. Some caution is therefore needed when attempting 391 to infer the actual genetic determinism of specific traits as the number of detected 392 QTL is likely to be smaller than the actual number of genes involved. QTL 393 experiments with a relatively small number of genotypes will mainly detect 394 major QTL with high allelic substitution effects and PEV. 395

QTL detection encompassed the whole range of traits and conditions described 396 above, focusing on short-term responses of physiological processes, or long-term 397 acclimation with the objective to investigate morphological adaptations. The 398 environmental conditions used for QTL detection, ranged from waterlogging and 399 flooding to total submergence. 400

## 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 404 and Mackill 1996; Nandi et al. 1997; Sripongpangkul et al. 2000; Xu et al. 2000; 405 Toojinda et al. 2003; GuangHeng et al. 2006; Hattori et al. 2007). All these 406 experiments detected a major QTL on chromosome 9 (see Chen et al. 2002, for a 407 detailed physical and genetic map of rice) and allowed the identification of the 408 sub-1 locus (Xu et al. 2006). QTL have also been detected for several species during 409 partial submergence and root hypoxia (Vantoai et al. 2001; Zheng et al. 2003; 410 Cornelious et al. 2005; Mano et al. 2005a, b; Cornelious et al. 2006; Githiri et al. 411 2006; Qiu et al. 2007; Parelle et al. 2007; Zhou et al. 2007a). Duration of water- 412 logging as well as the height of the water table were highly variable, ranging from a 413 few days (Qiu et al. 2007) to several weeks (Vantoai et al. 2001; Mano et al. 2005b). 414 and from few centimetres (Mano et al. 2005b; Qiu et al. 2007) to 10 cm above soil 415 surface (Vantoai et al. 2001; Cornelious et al. 2005). This diversity in experimental 416 procedures may have contributed to the large variability in the number and locali- 417 zation of the detected QTL (Table 12.2). Phenotyping after variable stress durations 418 and intensities may detect different tolerance processes and thus result in a QTL 419 detection that varies with environment. QTL detection is a statistical process, 420 whereby minor QTL with low allelic substitution effects and thus low PEV will 421 often be below the detection or significance limit. Parelle et al. (2007) detected a 422

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	IOT EACH EXPERIMENT (WHERE $n_i$ IS IN	e number of genotypes o	i une testeu 1	amuty)			
ť2.2	Article	Species	ni	Trait	$N_{\rm Q}$	LOD score	PEV
t2.3	Martin et al. (2006)	Iris	120	Survival	2	Ι	0.11 - 0.14
t2.4	Qiu et al. (2007)	Maize	288	Total dry weight	4	2.8-5.9	0.12 - 0.32
t2.5	Qiu et al. (2007)	Maize	288	Shoot dry weight	4	2.6-7.0	0.05 - 0.21
t2.6	Qiu et al. (2007)	Maize	288	Root length	7	2.5 - 3.5	0.04 - 0.07
t2.7	Qiu et al. (2007)	Maize	288	Root dry weight	2	2.7-2.9	0.04 - 0.05
t2.8	Qiu et al. (2007)	Maize	288	Plant height	б	2.7 - 3.2	0.05 - 0.07
t2.9	Mano et al. (2008)	Maize	195	Aerenchyma	5	1.5 - 3.4	0.04 - 0.09
t2.10	Mano et al. (2007)	Maize	141	Aerenchyma	9	1.7 - 4.9	0.07
t2.11	Mano et al. (2006)	Maize	178	Degree of leaf injury	1	4.4	0.03 - 0.15
t2.12	Mano et al. (2005a)	Maize	201	Adventitious root formation	2	3.9 - 5.1	0.09 - 0.1
t2.13	Mano et al. (2005a)	Maize	94	Adventitious root formation	1	6.5	0.25
t2.14	Mano et al. (2005b)	Maize	110	Adventitious root formation	б	3.2 - 5.1	0.1 - 0.21
t2.15	Parelle et al. (2007)	Oak	119	Hypertrophied lenticel Nr.	1	3.4	0.15
t2.16	Parelle et al. (2007)	Oak	100	Epinasty	5	7.2-15.2	0.08 - 0.12
t2.17	Parelle et al. (2007)	Oak	100	Hypertrophied lenticel dev.	1	2.8	0.1
t2.18	Zhou et al. (2007a)	Rice	282	Seedling height	1	2.3	0.04
t2.19	Zhou et al. (2007a)	Rice	282	Seedling emergence	7	2.1 - 3.9	0.04-0.07
t2.20	Zhou et al. (2007a)	Rice	282	Coleoptile emergence	2	2.3-4.9	0.04 - 0.08
t2.21	Zheng et al. (2006)	Rice	96	Seminal root length	1	2.6	0.12
t2.22	Zheng et al. (2006)	Rice	96	Total root dry weight	б	3.0–3.5	0.13 - 0.16
t2.23	Zheng et al. (2006)	Rice	96	Adventitious root number	5	0.53 - 2.98	0?-0.133
t2.24	Zheng et al. (2003)	Rice	96	Seminal root length	1	2.6	0.12
t2.25	Zheng et al. (2003)	Rice	96	Lateral root number	1	2.8	0.13
t2.26	Zheng et al. (2003)	Rice	96	Lateral root length	2	2.4	0.12
t2.27	Zheng et al. (2003)	Rice	96	Adventitious root number	4	2.5-4.6	0.11 - 0.20
t2.28	Xu and Mackill (1996)	Rice	169	Tolerance	1	36	0.69
t2.29	Toojinda et al. (2003)	Rice	172	Total shoot elongation	4	3.9–28.1	0.01 - 0.52
t2.30	Toojinda et al. (2003)	Rice	65	Total shoot elongation	б	6.2–27.3	0.24 - 0.74
t2.31	Toojinda et al. (2003)	Rice	172	Tolerance scrore	9	3.2-52.6	0.07 - 0.63
ť2.32	Toojinda et al. (2003)	Rice	65	Tolerance scrore	2	12.3–49.1	0.28-0.72

J. Parelle et al.





**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)

423 QTL for epinasty of which the PEV varied from 2.8% to 11.6% depending on the 424 observation date during permanent waterlogging. Furthermore, as discussed above, 425 the estimation of PEV and allelic substitution effect of QTL also depends on the 426 number of genotypes and of vegetative copies within genotypes. This, together with 427 the variations in environmental conditions, makes it difficult to compare QTL 428 among different experiments. However, comparing the position of major QTL 429 across experiments clarifies their significance across conditions and genotypes.

One major QTL detected in rice during total submergence is the the sub-1 locus 430 (Xu and Mackill 1996), showing five to seven times higher LOD scores and at least 431 two times higher PEV (Table 12.2) than QTL for any other adaptive trait despite the 432 similar ( $\pm 20\%$ ) number of genotypes involved in the different experiments, e.g.: 433 Mano et al. (2007, 2008) and Mano and Omori (2008) for aerenchyma; Mano 434 et al. (2005a, b) for adventitious roots; Mano et al. (2006) for leaf injury). Even 435 when taking into account that the estimated LOD and PEV depend on the experi-436 ment, the sub-1 locus stands out among all QTL detected for hypoxia tolerance. 437 This might be due to the fact that it controls internode growth, which is a trait 438 controlled probably by only few genes; whereas strategies of tolerance to water-439 logging are more complex, probably relying on multiple traits, and thus depending 440 on many, interacting genes. This would result in the detection of more QTL with 441

lower PEV. However, other than indicating effectively a stronger genetic determin- 442 ism, the high LOD scores for *sub-1* might also be due to the experimental control of 443 the stress intensity. The high water table present during total submergence experiments homogenizes the hypoxic stress among plants, thus reducing within-experi- 445 ment environmental variability; whereas hypoxic stress during waterlogging 446 experiments also depends on the homogeneity of the soil and the rooting density. 447 As oxygen diffusion in water is slow, the actual oxygen deficiency in the soil 448 depends on root and rhizospheric  $O_2$  consumption, which creates a gradient from 449 the soil surface to the root. This induces a large variability of the stress actually 450 perceived among plants within an experiment, with evident effects on the pheno- 451 types and on QTL detection. Indeed Parelle et al. (2007) detected QTL for dissolved 452 oxygen content in water in the vicinity of the roots of oaks submitted to water-453 logging, revealing a clear problem of stress control among plants, with genotypes 454 influencing their environment. 455

### 12.4.1.2 QTL Detection for Constitutive Traits of Tolerance

The most recently published experiments on maize (Mano et al. 2007, 2008) 457 detected QTL for aerenchyma formation, a key trait known to be highly related to 458 hypoxia tolerance. This was achieved with an inter-specific cross between two 459 species with different capacities of aerenchyma formation. They performed the 460 analysis only under control conditions, which avoids stress heterogeneity among 461 individuals (although not environmental variability). Aerenchyma formation is 462 usually enhanced under stress, thereby showing genotype x environment interactions and a different genetic determinism than under control conditions. This could 464 change allelic effects and PEV of the detected QTL. Genotype x environment 465 interaction could also be a cause for the small number of co-localised QTL between 466 the two different inter-specific crosses in the same experimental set-up: only one 467 out of seven detected QTL co-localized. 468

#### **12.4.1.3** Comparison with a Control Environment

QTL detection for induced adaptive traits does not necessarily require a control 470 treatment. Nevertheless, QTL detection for growth and productivity needs a com-471 parison between stress and control. This is necessary to distinguish constitutive 472 QTL that influence growth independently of the applied stress, and induced QTL 473 that control growth specifically in response to stress. Qiu et al. (2007) and Githiri 474 et al. (2006) compared the phenotypes expressed by the same genotypes growing in 475 water-logged versus control conditions. Githiri et al. (2006) computed the ratio of 476 seed production under waterlogging versus control, and used this ratio as a toler-477 ance index. However, QTL for ratios between stress and control are difficult to 478 interpret. This is mainly due to the fact that the condition that results in the highest 479

among-genotype variability dominates the statistical analysis. Three situations 480

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481 might occur: (1) the variability is larger under stress, the QTL is then related to 482 stress tolerance; (2) it is larger in controls (e.g. due to severe growth reduction under 483 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 484 treatments and it is difficult to conclude whether the detected QTL is related to 485 tolerance or not. Detecting QTL for each condition separately can provide support 486 for the interpretation. This was for example used by Qiu et al. (2007), who detected 487 QTL for the ratio of growth parameters between waterlogging and control as well as 488 489 for each treatment separately and used the resulting co-localisations to interpret QTL as either nonadaptive, constitutive or induced. Other than using stressed/ 490 control ratios as traits, both datasets can be used within the same statistical analysis, 491 such as in multi-environment QTL detection models (Jansen et al. 1995) which 492 allow a direct computation of QTL x environment interactions. This has been used 493 for example by Jermstad et al. (2003) to detect QTL in a factorial experimental 494 design using different winter chilling and spring flushing temperatures. Only one 495 application of multi-environment QTL detection is known to the authors in the case 496 of waterlogging. Parelle et al. (2007) recorded epinasty, root collar diameter and 497 leaf chlorophyll content in a Q. robur full-sib progeny and showed that the allelic 498 substitution effects of the detected QTL varied significantly during the 4 weeks 499 of waterlogging. This method described QTL by the temporal pattern of 500 the corresponding allelic substitution effect, and compared such patterns among 501 different QTL and traits. Interestingly QTL with correlated effect patterns were 502 503 dispersed over the whole genome, suggesting a polygenic determinism of tolerance 504 to hypoxia.

## 505 12.4.2 Major Loci Detected for Hypoxia Tolerance

506 One of the main tools for breeding crops for agriculture in areas submitted to 507 waterlogging or submergence is the detection of genomic regions or genes for 508 marker aided selection (Vartapetian 2005). On the other hand, QTL studies also aim 509 at elucidating the molecular mechanisms of hypoxia tolerance. However, only few 510 QTL experiments were performed with the aim to detect loci for which the genetic 511 diversity is effectively selected in natural populations. Table 12.1 summarizes the 512 traits and species for which QTL related to hypoxia response were detected.

# 513 12.4.2.1 *QTL* for Traits Submitted to Natural Selection Pressure 514 in Hypoxic Environments

515 Identifying candidate genes submitted to natural selection in water-logged or 516 flooded soils would advance our understanding of speciation processes in such 517 environments (Lexer et al. 2005). Martin et al. (2006) detected two QTL for 518 survival in an Iris family. Their experiment was performed under water-logged

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conditions in a common garden for 4 years. Another experiment was performed by 519 Parelle et al. (2007) who detected QTL for traits that are known to vary among 520 natural population of two sympatric, hybridising oak species (Q. robur and 521 Q. petraea). They detected two QTL for hypertrophied lenticel formation, five for 522 the level of epinasty, but none for adventitious root development. Loci identified 523 during these experiments could be a starting point in research strategies identifying 524 candidate genes. Such genes could then be screened for genetic variability in 525 natural populations. 526

### 12.4.2.2 QTL Detection for Breeding Purposes

Many QTL were detected for a large range of traits of interest for the maintenance 528 of productivity and growth under waterlogging (see Table 12.2 for details). Shoot 529 growth was the main indicator of productivity during hypoxia, and QTL were 530 detected for shoot biomass by Qiu et al. (2007) or shoot height by GuangHeng 531 et al. (2006) and Qiu et al. (2007). Vantoai et al. (2001) used shoot growth during 532 stress as a tolerance index, considering that eliminating the growth before stress 533 would detect induced rather than constitutive QTL. As the root system is directly 534 affected by hypoxia, some authors use it as an indicator of tolerance, for example 535 Qiu et al. (2007) detected QTL for root length under hypoxic condition, or Zheng 536 et al. (2006) for total root biomass. QTL detected for these traits might be used for 537 marker aided selection or to produce inbred lines to improve crop performance 538 under hypoxia (Vantoai et al. 2001), without necessarily having detailed informa-539 tion 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 542 hypoxia-induced morphological traits: 11 QTL were identified for aerenchyma 543 formation in maize (Mano et al. 2007, 2008) and two QTL for hypertrophied 544 lenticel formation in oaks (Parelle et al. 2007). Six QTL for adventitious root 545 development were detected in maize (Mano et al. 2005a, b), but none in *Q. robur* 546 despite a visible development of such roots. This lack of genetic determinism for 547 adventitious root development in oak might be due to heterogeneity of soil hypoxia, 548 which dominated the phenotypic variance of this trait, and therefore diluted the 549 genetic variance (Parelle et al. 2007). 550

To characterise the tolerance of rice to total submergence, internode elongation 551 was used to quantify the capacity of quiescence or stress escape. In consequence, 552 this trait allowed the detection of two types of loci related to those two strategies. 553 This can be used in QTL detection studies when specific crosses are used, either 554 combining two genotypes with different strategies or a tolerant and a non tolerant 555 genotype. Indeed, the sign of the allelic substitution effect, combined with the 556 knowledge of the strategy developed by the parents allowed to clearly attribute 557

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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

t3.1 **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

t3.9 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 558 et al. 2003; Nemoto et al. 2004; Tang et al. 2005; Hattori et al. 2007; Kawano et al. 559 2008) detected QTL related to the escape strategy of different rice varieties, and 560 561 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 562 which QTL were detected at the sub-1 locus. Sripongpangkul et al. (2000) and 563 Hattori et al. (2007) performed multiple trait phenotyping of the early elongation 564 ability, where the constitutive diversity included in each trait differed, whereas 565 stress responses relating to the same tolerance mechanism would result in co-566 567 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 568 step for breeding was the successful introgression of the sub-1 locus into a rice variety 569 of economic importance. Siangliw et al. (2003) crossed three tolerant varieties of rice, 570 containing the *sub-1* allele conferring tolerance by quiescence strategy, with the 571 hypoxia intolerant Thai jasmine rice. In the hybrid families, QTL were detected for 572 hypoxia tolerance at the *sub-1* locus and the alleles related to quiescence were always 573 from the tolerant parent. Introgression increased survival from 1.6% in Thai jasmine 574 to 23%-31% in the hybrid families. This example shows how the detection of a QTL 575 for tolerance can be used directly for breeding purposes. 576

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.

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The *sub-1* locus was not only detected in F13A, but also in other tolerant 584 varieties. For example Sripongpangkul et al. (2000) detected a QTL for elongation 585 ability on the *sub-1* locus in a F8 RIL cross from two *indica* cultivars; the tolerant 586 parent conferring the allele for a, faster elongation ability. This locus therefore 587 seems to be also involved in the escape strategy developed by some deep-water 588 varieties of rice. However, the main QTL for the fast elongation capacity was 589 detected on a different chromosome than *sub-1* (Tang et al. 2005; Hattori et al. 590 2007, 2008).

Candidate gene approaches were used to identify the gene(s) beneath the *sub-1* 592 locus. Ruanjaichon et al. (2004) first mapped a small GTP-binding protein, belong-593 ing to a family known to be involved in signal transduction pathways and Kottapalli 594 et al. (2006) identified 1,473 (putative) genes. However the large confidence 595 interval for the position of the QTL for sub-1 (6,4 cM for Kottapalli et al. 2006) 596 did not allow the identification of the gene(s) responsible for submergence toler-597 ance. It was finally the sequencing of the rice genome (International Rice Genome 598 Sequencing Project 2005), the construction of a high resolution genetic map 599 (Harushima et al. 1998; Xu et al. 2000) and the comparison of genetic and physical 600 maps (Kamolsukyunyong et al. 2001; Chen et al. 2002) that allowed the identifica- 601 tion of the *sub-1* cluster of genes (Xu et al. 2006). To date, the physical structure of 602 this locus is well known (see Fukao et al. 2006 and Xu et al. 2006 for details). This 603 locus contains 13 genes, including three ethylene response factors called Sub1-A, 604 Sub1-B and Sub1-C. Sub1-A is present only in O. sativa ssp indica, including the 605 tolerant variety F13A (Xu et al. 2006; Fukao et al. 2009). This gene originates 606 probably from the duplication of the Sub1-B gene, as the two genes display a large 607 sequence homology, and as the presence of the Sub1-A gene was correlated with 608 variation of Sub1-B alleles (Fukao et al. 2009). Two alleles of Sub1-A, have been 609 reported: the Sub1-A-1 and the Sub1-A-2. These alleles were correlated with 610 variation of alleles of Sub1-C (Fukao et al. 2009). The tolerant variety F13A 611 possesses the Sub1-A-1 allele and the corresponding alleles of the Sub1-C and 612 Sub1-B. Recombinant crossing experiments among the three genes (Xu et al. 2006; 613 Septiningsih et al. 2009) demonstrated that variation of the two alleles of the 614 Sub1-A locus modify the submergence tolerance of rice, independently of the 615 effect of the alleles present in Sub1-B and Sub1-C. This suggests that the QTL is 616 controlled by the allelic effect of one single gene, of which only two states were 617 detected: presence / absence of the Sub1-A-1 allele or the presence / absence of the 618 entire gene. Introgression of the Sub1-A-1 allele into other species than rice could 619 improve productivity of crops under flooding. The Sub1-A-1 allele induces the 620 quiescence strategy resulting from the inhibition of internode elongating (Toojinda 621 et al. 2003). Actually the Sub1-A-1 allele inhibited the effects of the Sub1-C gene 622 on elongation initiation in response to ethylene (Fukao et al. 2006). However, it is 623 far from being clear whether the introgression of the Sub1-A-1 allele into other 624 species actually confers a larger tolerance to total submergence, as interactions with 625 other tolerance strategies need to be taken into account. 626

It is interesting to notice that the three *sub1* genes (A, B, and C) are ethylene 627 response factors. Ethylene is known to be involved in a large number of hypoxia 628



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.

## 633 12.5 Conclusions

Genetic diversity has been shown among populations or within mapping families, 634 635 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 eluci-636 dated for only a small number of traits and even less gene candidates have actually 637 been tested. At the enzyme level, some diversity was detected only for ADH. 638 However, no signal transduction pathway has been put forward and related to the 639 640 observed genetic differences. Further, the actual effect of genetic differences in ADH on survival and fitness in natural populations still lacks experimental support. 641 The correlation between the observed genetic diversity of short term metabolic 642 adjustments to hypoxia and long term morphological adaptations needs further 643 investigation. The QTL detected for survival or traits known to vary among natural 644 populations could be starting points for gene candidate approaches. Such gene 645 candidates could then be screened for natural genetic variability, thereby generating 646 knowledge on the adaptability of populations, especially with respect to environ-647 mental changes. 648

The major challenge for future QTL detection for traits conferring hypoxia 649 tolerance is the definition of integrative traits (1) indicating different tolerance 650 strategies and (2) well suited to high-throughput phenotyping required for quanti-651 tative genetic analyses. In addition to the sub-1 locus, a large number of minor QTL 652 have been detected. The combination of several favourable alleles will determine 653 the tolerance of an individual. However to decompose these processes, future 654 approaches should combine large scale QTL experiments using complex traits 655 and detailed studies on selected genotypes to decompose overall tolerance into 656 elementary components. 657

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# Author Queries

Chapter No.: 12

Query Refs. Details Required		Author's response
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AU2	Following reference is not cited in text: Levy et al. (1999)	