New Phytologist

Review

Tansley review

The genetics of drought tolerance in conifers

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Received: *20 March 2017*
Accepted: *27 July 2017*

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Contents

New Phytologist (2017)
doi: 10.1111/nph.14774

Key words: association, conifer, drought tolerance, gene expression, genetics, genomic, local adaptation, provenance.

Summary

As temperatures warm and precipitation patterns shift as a result of climate change, interest in the identification of tree genotypes that will thrive under more arid conditions has grown. In this review, we discuss the multiple definitions of 'drought tolerance' and the biological processes involved in drought responses. We describe the three major approaches taken in the study of genetic variation in drought responses, the advantages and shortcomings of each, and what each of these approaches has revealed about the genetic basis of adaptation to drought in conifers. Finally, we discuss how a greater knowledge of the genetics of drought tolerance may aid forest management, and provide recommendations for how future studies may overcome the limitations of past approaches. In particular, we urge a more direct focus on survival, growth and the traits that directly predict them (rather than on proxies, such as water use efficiency), combining research approaches with complementary strengths and weaknesses, and the inclusion of a wider range of taxa and life stages.

I. Introduction

Climate change is increasing water stress in many areas as a result of increasing evaporative demand, altered precipitation and earlier snowmelt (Ryan, 2011; IPCC, 2013). Moderate drought is a common cause of reduced growth and increased mortality (van Mantgem & Stephenson, 2007; McDowell *et al.*, 2010), whereas severe droughts have led to mass mortality (Breshears *et al.*, 2005; Allen *et al.*, 2010). Differences in drought susceptibility between tree species and populations have both environmental and genetic components. There is evidence of local adaptation to climate in many trees despite high gene flow (Aitken *et al.*, 2008). Populations often do not respond equally to a given climate (Rehfeldt *et al.*, 2014). To better manage tree populations, we need to understand the relationships between tree genotype and phenotype (Gailing *et al.*, 2009; Neale & Kremer, 2011) and between phenotype and ecological function (Tyree & Ewers, 1991; Sevanto *et al.*, 2014; Montwé *et al.*, 2015; Reinhardt *et al.*, 2015). The relative importance of drought response plasticity vs genetic differences is still largely unknown, as are details of which traits are most important for drought tolerance and what genes underlie them.

Here, we focus on the genetics of drought tolerance in conifers. Although some general principles apply to both angiosperms and gymnosperms, there are significant differences. Gymnosperms

are generally more drought resistant as a result of lower stomatal sensitivity to vapor pressure deficit (VPD) and more cavitation-resistant xylem (Carnicer *et al.*, 2013). Conifer xylem is made up entirely of tracheids, whereas angiosperms may produce both tracheids and wide vessels, which have higher hydraulic conductivity, but a smaller margin of safety with regard to xylem pressures (Carnicer *et al.*, 2013). In addition, angiosperms have more complex anatomical responses to drought, such as changes in vessel connectivity (Brodribb *et al.*, 2012). Moreover, much research attention has been given to drought tolerance in conifers because they are a dominant component of many arid zone forests. We first address the definitions of 'drought tolerance' and the physiological mechanisms involved. We then describe three major methods that have been used to study drought tolerance genetics, and review the major findings to date. Finally, we discuss how genetic tools may aid forest management, and needs for future research. In particular, we recommend the combination of complementary methods, and the broadening of the range of phenotypes, taxa, life stages and timespans examined.

II. Drought tolerance

1. Definition of 'drought tolerance'

A basic definition of drought tolerance is the ability to survive, and sometimes grow, during periods of water shortage. Survival and growth are often correlated, with trees exhibiting a history of below average growth or abrupt decreases in growth having higher mortality (Wyckoff & Clark, 2002; van Mantgem *et al.*, 2003; Das *et al.*, 2007; Huang *et al.*, 2015). Because of this, and because tree genetics studies are often motivated by wood production, some studies define drought tolerance as growth maintenance (e.g. Orwig & Abrams, 1997; Eilmann *et al.*, 2010). However, drought length and duration can affect the growth–mortality relationship. For instance, growth plasticity (high growth in good conditions, low in bad) may be adaptive in variable environments (Santos-Del-Blanco *et al.*, 2013). Populations adapted to extended drought often exhibit conservative resource use strategies, resulting in slower growth rates, even in favorable conditions (Correia *et al.*, 2008; Herrero *et al.*, 2013; Kerr *et al.*, 2015; Montwé *et al.*, 2015).

Drought tolerance can be broken down into several categories. 'Drought avoidance' strategies (e.g. deep roots or stomatal adjustments) reduce exposure to drought stress (McDowell *et al.*, 2008). However, it is unclear to what degree trees truly avoid drought stress relative to plants that go dormant. 'Drought resistance' is the ability to withstand drought exposure, whereas 'drought resilience' is a measure of how quickly a tree can resume normal growth when conditions improve (Lloret *et al.*, 2011; Eilmann & Rigling, 2012).

2. Mechanisms of drought tolerance

The physiological responses of trees to drought have been reviewed elsewhere (Sala *et al.*, 2010; McDowell, 2011; Anderegg *et al.*, 2012; Choat *et al.*, 2012; Carnicer *et al.*, 2013; Sevanto *et al.*, 2014). We include a brief discussion here to highlight some of the traits and processes that are involved in drought avoidance, resistance and resilience.

Avoidance of hydraulic failure and decreased cellular water potential Conifers manage tissue water potential in two main ways: isohydric trees close stomata to maintain water potential, whereas anisohydric species allow water potential to drop (Tardieu & Simonneau, 1998) (Fig. 1a,b). Isohydric trees use increasing abscisic acid (ABA) concentrations as a signal to keep stomata closed, whereas anisohydric trees use low leaf water potential itself as a signal to close stomata (Brodribb *et al.*, 2014). Anisohydric conifers include many Cupressaceae and some Taxaceae (McDowell *et al.*, 2008; Brodribb *et al.*, 2014).

Xylem architecture affects how changes in stomatal conductivity influence cavitation risk, and anisohydric trees tend to have xylem that is more cavitation resistant (McDowell *et al.*, 2008). Wider tracheids increase conductivity and the risk of hydraulic failure (Sperry *et al.*, 2006), whereas those with smaller inter-tracheid pits or more lignified walls are less vulnerable (Sperry *et al.*, 1988; Tyree & Ewers, 1991). The reduction of leaf area with branch die-back, reduced needle number or smaller needles (Cinnirella *et al.*, 2002; Eldhuset *et al.*, 2013) can also reduce water loss. Anisohydric species often exhibit branch die-back during drought, whereas isohydric trees typically retain a full canopy until death (Koepke *et al.*, 2010) (Fig. 1a,b).

Some conifer species can refill xylem following cavitation. This is thought to be an energy-intensive process that depends on carbon reserves (Carnicer *et al.*, 2013). This may explain why drought-stressed trees can exhibit lower refilling capability (Cinnirella *et al.*, 2002). *Picea abies* refills freezing-cavitated xylem before soils have thawed by taking up water through its needles (Mayr *et al.*, 2014). This could explain why other conifers can refill xylem in the absence of positive root pressures, unlike co-occurring angiosperms (Sperry *et al.*, 1994). However, refilled xylem may be less resistant to future drought stress, a characteristic known as 'cavitation fatigue' (Hacke *et al.*, 2001).

Prevention of cellular damage when water content drops Loss of water potential in cells is associated with cell turgor loss, denaturation of proteins and changes in membrane fluidity. To avoid cellular damage, plants synthesize molecules that act as osmotic balancing agents. These reduce cellular solute potential, and may increase turgor at lower water potentials. In addition, hydrophilic compounds can prevent the membranes from leaking (Valliyodan & Nguyen, 2006; Yuxiu *et al.*, 2007). Other compounds stabilize proteins or detoxify reactive oxygen species. These protective molecules include proteins such as chaperonins and dehydrins (Hamanishi & Campbell, 2011), the amino acid proline (Hayat *et al.*, 2012) and various carbohydrates (Valliyodan & Nguyen, 2006; Lorenz *et al.*, 2011). We hypothesize that protective molecules may be produced earlier during a drought in anisohydric species because leaf water potential drops more quickly (Fig. 1c).

Management of carbon reserves As a result of reduced stomatal conductance (g_s), oxidative damage and other factors, photosynthetic rates and chlorophyll concentrations often decline during drought (Dubos *et al.*, 2003; Watkinson *et al.*, 2003). Therefore, in addition to protecting cells from damage, increased allocation to

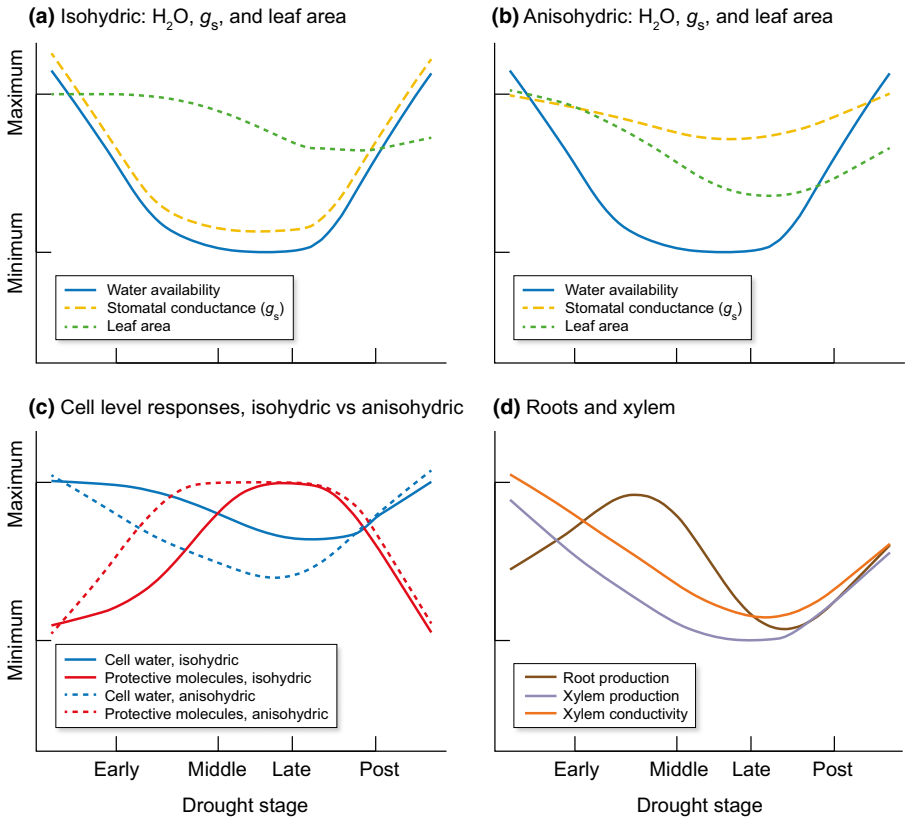

Fig. 1 Hypothetical drought responses. (a) In isohydric trees, stomatal conductance is reduced when water availability is low to avoid water loss, but leaf area is largely maintained. (b) In anisohydric trees, stomatal conductance is maintained, but leaf area may be reduced. (c) As a result, cell water content drops more strongly and earlier in anisohydric species. This could necessitate earlier expression of protective molecules. (d) Trees sometimes produce more roots in response to early or mild drought, but prolonged drought can reduce root growth. Xylem production and the conductivity of the xylem tend to decline during drought.

nonstructural carbohydrates (NSCs) may help to avoid carbon starvation by keeping energy resources in easily mobilizable forms. The patterns of change in overall NSCs and starches seem to differ between species and drought length and severity (Oberhuber *et al.*, 2011; Adams *et al.*, 2013; Anderegg & Anderegg, 2013; Reinhardt *et al.*, 2015). Clear evidence of death as a result of carbon depletion is still lacking (McDowell & Sevanto, 2010; Sala *et al.*, 2010). However, carbon storage and allocation patterns do vary under drought stress (Galiano Pérez *et al.*, 2017), demonstrating implications of water limitation on carbon availability.

Influences of anatomical structures, individual plasticity and drought legacy effects Root and branch patterns can influence the degree of water stress experienced. The number of branches and leaves affects total transpiration. Rooting depth affects access to deep soil water and is probably crucial for seedlings (Cregg & Zhang, 2001; Olmo *et al.*, 2014) as well as adult trees in areas with seasonal drought (O'Brien *et al.*, 2017). Deep roots may also redistribute water from deep to shallow soils (Horton & Hart, 1998; Brooks *et al.*, 2002). More small diameter roots, with high surface area : volume and a lower vulnerability to cavitation, may aid drought resistance (Phillips *et al.*, 2016).

Structural changes can have long-lasting effects. Decreasing soil moisture can induce greater root production, but extended drought reduces root mass (Eldhuset *et al.*, 2013) (Fig. 1d), which limits responsiveness to precipitation pulses (Plaut *et al.*, 2013). Lumen width and cell wall thickness of tracheids are plastic, with those produced in moist seasons and years generally being wider, more numerous and thinner walled than those produced in dry periods (Eldhuset *et al.*, 2013; Xu *et al.*, 2014) (Fig. 1d). Xylem is often functional for multiple years (Eilmann *et al.*, 2010; Mayr *et al.*, 2014; Xu *et al.*, 2014; Kimak & Leuenberger, 2015), and so current drought responses can affect water transport during future drought.

The production of protective molecules typically drops soon after normal water potential is restored (Lorenz *et al.*, 2011). However, transcriptional and physiological 'memory' in stomatal guard cells has been observed, with stressed plants maintaining smaller stomatal apertures when re-watered (Virlouvet & Fromm, 2015). There may also be 'legacy effects' on NSC production (Villar-Salvador *et al.*, 1999) and traits such as growth and xylem anatomy (Anderegg *et al.*, 2015). Plants that quickly return to normal could gain a growth advantage. In areas in which recurring drought is common, however, we hypothesize that this memory effect reduces mortality risk.

There are multiple traits involved at different stages of the drought response (Fig. 2). Stomatal control and patterns of root and shoot growth affect the degree to which a plant avoids drought stress. These traits plus xylem morphology, protective molecule production, changes in carbohydrate metabolism and pathogen defenses influence drought resistance. Finally, the recovery rate of photosynthesis and other processes, the degree of persistent changes in structure and the ability to refill xylem affect drought resilience. In the next two sections, we first review the methods used to date to examine genetic controls on ecologically important traits, and then explore how these methods have been and can be leveraged to test for genetic variation in, and identify the genetic basis of, the traits and processes addressed above.

Physiological studies		Genetic studies			
Key traits/ responses	Stages	Expression	Provenance	Genome scan/ G2E	QTL/G2P
Stomatal control (ABA pathway)	Avoidance Resistance Resilience	↑	↑?	Δ	Δ
Shoot growth	Avoidance Resistance Resilience	↓? (cell division)	↓?	Δ? (cell division)	Δ? (cell division)
Protective molecules (e.g. dehydrins)	Resistance	↑	?	Δ	Δ
Pathogen defense	Resistance	↑	?	Δ	?
Nonstructural carbohydrates	Resistance Resilience	Δ? (carbohydrate metabolism)	?	Δ? (carbohydrate metabolism)	?
Root growth	Avoidance Resistance Resilience	?	↑?	?	?
Xylem morphology	Resistance Resilience	?	?	Δ? (cell wall)	Δ? (cell wall)
Xylem refilling	Resilience	?	?	?	?

Fig. 2 Connections between traits or responses identified in physiological studies as important for drought tolerance and genetic studies. The latter include shifts in gene expression under drought, differences between source populations (provenances) from dry vs wet environments, differences in alleles across environmental gradients (genome scan or G2E association), and differences in alleles associated with a phenotype (QTL or G2P association). Arrows indicate the direction of difference between dry vs wet, environment or provenance; Δ indicate a difference where the direction of change is unclear. Question marks indicate where a relationship is unclear, either because it has not been investigated, or because it is unclear how or if the genes identified relate to the process or trait. Narrow arrows paired with question marks indicate a directional change in something that might be related to the process or trait.

III. Investigation of the genetic basis of drought tolerance: three main approaches

1. Gene expression/transcriptome studies

Gene expression or transcriptome studies examine changes in the amount of RNA transcripts to identify genes that are upregulated or downregulated under different conditions. Changes in the amount of a gene product (e.g. chaperone protein) can result in different phenotypic responses, even if all individuals have the same gene sequence. Such changes are responsible for plasticity, and may involve temporary or heritable epigenetic modifications (Bräutigam *et al.*, 2013).

Gene expression studies may involve a variety of techniques, but most recent studies have used microarray chips – DNA probes to which cDNA or RNA hybridize, resulting in fluorescence (Watkinson *et al.*, 2003; Lorenz *et al.*, 2011) – or cDNA sequencing (e.g. RNAseq) (Lorenz *et al.*, 2006; Behringer *et al.*, 2015). The latter avoids the need for probe and microarray design and can survey whole novel transcriptomes (Wang *et al.*, 2009). Real-time quantitative polymerase chain reaction (qPCR) is highly sensitive, but is most often used to target specific candidate genes (Perdiguero *et al.*, 2012a; Velasco-Conde *et al.*, 2012) or to confirm a subset of expression changes (Perdiguero *et al.*, 2012b; Behringer *et al.*, 2015). All techniques are sensitive to which tissues are sampled at what time (Fossdal *et al.*, 2007; Yeaman *et al.*, 2014). Moreover, unless expression responses in different genotypes or populations are explicitly compared, this approach does not address local adaptation.

2. Provenance studies

Provenance or common garden studies, where seedlings from many different sources are planted in a common environment, began to reveal heritable differences between tree populations long before the availability of genetic marker data (Callaham & Liddicoet, 1961; Rehfeldt *et al.*, 1984; Rehfeldt, 1991; Correia *et al.*, 2008). Provenance studies established in the mid-20th century to identify seed zones for replanting or highly productive genotypes have been re-purposed to investigate potential responses to climate change (Schmidtling, 1994; Rehfeldt *et al.*, 2002, 2014; Wang *et al.*, 2006; O'Neill *et al.*, 2008; Leites *et al.*, 2012). Many recent studies have also used seedling common gardens (see Section IV.2). Studies conducted across multiple sites, or incorporating multiple treatments, can estimate the plasticity of traits, allowing the fitting of transfer functions that predict performance based on source and planting environments (Wang *et al.*, 2006; Leites *et al.*, 2012; Rehfeldt *et al.*, 2014). However, such studies do not reveal *which* genes are responsible for observed differences unless paired with other techniques. It should be noted that there is usually substantial variation within tree populations (Aitken *et al.*, 2008). The third set of approaches can be used to investigate the causes of heritable variation between populations and individuals.

3. Quantitative trait locus (QTL), genome scan and genotype association studies

These approaches aim to identify genes or genomic regions related to a trait or to adaptation along environmental gradients. QTL

studies are a classic way to identify the loci involved in continuous trait variation. However, although QTLs for a number of traits have been identified in trees, this approach has had limited success for a variety of reasons, many of which are reviewed in González-Martínez *et al.* (2006). For instance, a great deal of time and space is needed to cross parental tree lines and raise a sufficient sample size of progeny. Conifers also have very large genomes with low linkage disequilibrium (LD) and, without enough genetic markers available, most QTLs are undetectable (Neale & Kremer, 2011). In addition, high-resolution genetic/physical maps or positional cloning is needed to identify causal genes/mutations (Aitken *et al.*, 2008; Neale & Kremer, 2011; de Miguel *et al.*, 2014).

By contrast, genome scan and association studies make use of large numbers of newly available markers (e.g. single nucleotide polymorphisms, SNPs), and are carried out in highly diverse outcrossing natural populations (González-Martínez *et al.*, 2006). Genome scans identify loci that differ more or less between populations than expected by chance (Eveno *et al.*, 2008; Namroud *et al.*, 2008; Prunier *et al.*, 2011). For instance, outlier F_{st} values can be used to infer the type of selection: balancing selection results in low F_{st} and shared alleles, and divergent selection in high F_{st} with segregated alleles. Genome scans can also identify patterns suggestive of a selective sweep. These studies do not automatically provide information about which, if any, environmental variables are responsible for the pattern. One can test whether patterns of differentiation match an environmental gradient, but this is necessarily a *post-hoc* interpretation (Eckert *et al.*, 2010a).

Association studies use a regression approach to identify loci in which genetic variation is associated with variation in trait values or home environment. Such analyses can be carried out at the individual (genotype) or population (allele frequency) level. Genotype-to-environment (G2E) association studies identify loci that vary along environmental gradients (Eckert *et al.*, 2010a; Prunier *et al.*, 2011; Frichot *et al.*, 2013). An association between an SNP and aridity, for example, suggests that the gene or its regulatory region affects performance in wet vs dry environments. This does not reveal *how* the locus affects phenotype, and careful interpretation is needed as a result of correlation between climatic variables. Genotype-to-phenotype (G2P) association studies identify loci correlated with a particular phenotype (Neale & Savolainen, 2004; Holliday *et al.*, 2010; Cumbie *et al.*, 2011), but the phenotype may or may not be relevant for fitness in the field.

Most association studies in conifers to date have used SNPs in a limited number of candidate genes (Gonzalez-Martinez *et al.*, 2005; Aitken *et al.*, 2008; Holliday *et al.*, 2010). This ensures that genes suspected of involvement are surveyed, but limits the ability to identify additional loci. However, with the decreasing cost of sequencing, approaches that generate large numbers of SNPs are increasingly being used for genome-wide association studies (GWAS). One set of approaches, including RAD-seq and genotyping-by-sequencing (GBS), involves the use of restriction enzymes to cut and sequence a small subset of the genome (Elshire *et al.*, 2011; Poland & Rife, 2012; Andrews *et al.*, 2016). This can produce tens of thousands of SNPs with high coverage (Chen *et al.*, 2013; Karam *et al.*, 2015; Pan *et al.*, 2015). Many of these SNPs will be in noncoding regions, which is good for the potential discovery of regulatory regions, but can limit the number of gene associations detected. Another approach involves the creation of a transcriptome or full genome sequence for a species, and the development of probes for all or most of the putative genes to identify SNPs (Cokus *et al.*, 2015; Jaramillo-Correa *et al.*, 2015; Gugger *et al.*, 2016). This approach can also yield useful gene expression data if multiple tissue types or treatments are included in the development of the transcriptome (Yeaman *et al.*, 2014).

IV. Conifer drought tolerance genetics: what we know

1. Changes in gene expression during drought

Overall changes in gene expression in drought-stressed conifer seedlings Most drought gene expression studies in conifers have focused on pine seedlings, with a few investigating other Pinaceae genera (Table 1, column 3). The direction of expression responses to the environment, including dry conditions, is highly conserved (74% of orthologs) between *Pinus contorta* and *Picea glauca* × *engelmannii*, even though average expression levels often differ (Yeaman *et al.*, 2014). It is unclear whether this is true across conifer families. No expression studies have focused on adult drought responses.

The methods used to induce drought stress vary. Studies have withheld water for a specified period (Perdiguero *et al.*, 2012a), until soil moisture reached a threshold (Velasco-Conde *et al.*, 2012; Eldhuset *et al.*, 2013) or needles wilted (Lorenz *et al.*, 2011), or needle water content declined to a certain level (Watkinson *et al.*, 2003; Behringer *et al.*, 2015). Some have used chemically induced water stress (Dubos *et al.*, 2003; Perdiguero *et al.*, 2012b). Caution must therefore be used in interpreting differences across studies, as these could be methodological artifacts (Watkinson *et al.*, 2003).

Genes related to signaling and gene transcription are frequently upregulated in drought-stressed seedlings. Changes in signal cascades must precede changes in their targets, and such expression shifts often occur within the first week of drought stress. Those in the ABA pathway are well represented (Fig. 2; Table 1). In addition to being involved in stomatal closure, ABA signaling can affect shoot growth and water uptake (Parent *et al.*, 2009; Hamanishi & Campbell, 2011). However, there are also ABA-independent pathways in most taxa, which may use leaf water potential as a signal (Valliyodan & Nguyen, 2006; Hamanishi & Campbell, 2011; Brodribb *et al.*, 2014). Upregulation of genes in the ethylene pathway (Lorenz *et al.*, 2011; Perdiguero *et al.*, 2012b) could be related to reduced shoot growth or leaf area (Carnicer *et al.*, 2013).

Genes related to protective molecules are also frequently upregulated (Fig. 2; Table 1). Late-embryogenesis-abundant (LEA) proteins, named for their role in seeds, appear to stabilize proteins and membranes and prevent protein aggregation (Close, 1996; Goyal *et al.*, 2005). Dehydrins, a subgroup of LEAs, often protect against drought stress, although some are induced by other abiotic stresses (Yuxiu *et al.*, 2007; Perdiguero *et al.*, 2012a; Velasco-Conde *et al.*, 2012). Heat shock proteins, detoxification enzymes and genes in the synthesis and transport pathways of osmoprotective carbohydrates and proline may also be upregulated

Table 1 Gene categories linked to drought responses in conifers by four different approaches

Functional category	Subcategory	Expression studies	Genome scans	G2E association	QTL or G2P association
Protective proteins	Dehydrins	ABAL[1]; PIPI[14,17]; PITA[11,12,18]	PIPI[8]		PITA[9]
	Other LEAs	ABAL[1]; PIPI[15]; PITA[11,12,18] (+/−)	PIMA[16]		
	Heat shock proteins	PIPI[15]; PITA[12,18]		PICE & PIMU[13]	PIPI[2]
	Chaperones & their regulators			PITA[5]	PIPI[2]
Osmoprotective carbohydrates	Transport	PIPI[15]; PITA[12]			PILA[7]
	Synthases	ABAL[1]; PIPI[15]; PITA[12]			
Proline synthesis		PITA[12]			
Detoxification	Aldehyde dehydrogenase	PIPI[15]		PIPI[10]	
	Oxidative stress defense	PIPI[15]; PITA[12]		PITA[5]	PIPI[2]; PITA[9]
	Other	ABAL[1]; PITA[18]			
Signaling & gene transcription	ABA pathway	ABAL[1] (+/−); PITA[11,12]	PIPI[8]	PICE[13]	PITA[3]
	Ethylene	PIPI[15]; PITA[12]		PIPI[10]	PIPI[2]
	Various transcription factors	ABAL[1] (+/−); PIPI[15]; PITA[12]	PIMA[16]	PIPI[10]	PIPI[2]; PITA[9]
	Kinases	ABAL[1]; PIPI[4,15] (−)		PILA[7]; PIMU[13] PITA[5]	PIPI[2]
	Non-kinase signal tranduction	ABAL[1]; PIPI[15]		PICE[13]	PIPI[2]
	Histones	PITA[12]			
	Methylation	PITA[11]	PIPI[8]		
	Other	PIPI[4,15] (+/−); PITA[11]		PILA[7]; PITA[5]	PIPI[2]; PITA[3]
Cell wall construction		ABAL[1] (−); PIPI[4] (−); PITA[11,12] (−)	PIPI[8]	LADE & PIMU[13]; PIPI[10]	PIPI[2]; PITA[9]
Cytoskeleton		ABAL[1] (−); PITA[18]		PIPI[10]; PITA[5]	
Growth/cell division or expansion		PIPI[4,15] (−); PITA[12]		PITA[5]	PIPI[2]
Cation/H^+ transporters		ABAL[1]		PIPI[10]; PITA[5]	PIPI[2]
Carbohydrate metabolism	Carbohydrate synthesis	ABAL[1] (−); PIPI[4,15] (+/−)			
	Breakdown	ABAL[1]; PIPI[15]; PITA[12,18] (+/−)		LADE & PIMU[13]	PIPI[2]
	Other	PIPI[4] (+/−); PITA[12] (−)		LADE & PIMU[13] PILA[7]	
Biotic stress defense		ABAL[1]; PIPI[4,15]; PITA[11,12]		PILA[7]; PITA[5]	
Protein handling/ breakdown	Ubiquitin pathway	ABAL[1]	PIMA[15]	PIMU[13]; PITA[5,6]	PIPI[2]
	Lysosome	PIPI[4] (−)		LADE[13]	
	Proteasome	PIPI[15]		PICE[13]	
Translation	Ribosomes or mRNA processing tRNAs			LADE & PICE[13]; PIPI[10] ABAL[13]	
	Translation initiation	PIPI[4]			
Photosynthesis	Photosystems	PIPI[4] (−)		PIPI[10]; PITA[5]	PIPI[2]
	Photorespiration	PIPI[4] (+/−)			
	Rubisco	PIPI[4] (−); PITA[18]			
	Other chloroplast proteins				PIPI[2]
Lipid metabolism		ABAL[1]; PIPI[15]; PITA[12] (+/−)			PIPI[2]
Transport	Carbohydrate	ABAL[1]		PICE[13]; PIPI[10] PITA[5]	
	Water (aquaporins)	ABAL[1]; PITA[12]			PIPI[2]
	Protein	PIPI[4] (−)			
	Other	PIPI[15]			
Amino acid metabolism		ABAL[1]; PIPI[15]; PITA[18] (+/−)			PIPI[2]
Mitochondrial proteins		PITA[12]		PIPI[10]; PITA[5]	PIPI[2]; PITA[3]
Heavy metal binding		PIPI[4] (−)			
Pollen				PITA[5]	
Miscellaneous metabolism		ABAL[1] (+/−); PITA[11]		PITA[5]	

(−) In expression studies indicates downregulation as opposed to upregulation, whereas (+/−) indicates a difference between tissues or genes in the category. ABA, abscisic acid; G2E, genotype-to-environment; G2P, genotype-to-phenotype; LEA, late embryogenesis abundant; QTL, quantitative trait locus; WUE_i, intrinsic water use efficiency. Species codes: ABAL, *Abies alba*; LADE, *Larix decidua*; PIAB, *Picea abies*; PICE, *Pinus cembra*; PILA, *Pinus lambertiana*; PIMA, *Picea mariana*; PIMU, *Pinus mugo*; PIPI, *Pinus pinaster*; PITA, *Pinus taeda*.

[1]Behringer *et al.* (2015) (qPCR-validated loci with 3–10-fold change in expression); [2]de Miguel *et al.* (2014) (QTLs for traits measured during drought stress); [3]Cumbie *et al.* (2011); [4]Dubos *et al.* (2003); [5]Eckert *et al.* (2010a) (loci with Bayes Factor > 100); [6]Eckert *et al.* (2010b); [7]Eckert *et al.* (2015); [8]Eveno *et al.* (2008); [9]Gonzalez-Martinez *et al.* (2008); [10]Jaramillo-Correa *et al.* (2015); [11]Lorenz *et al.* (2006); [12]Lorenz *et al.* (2011) (genes with greatest change in expression); [13]Mosca *et al.* (2012); [14]Perdiguero *et al.* (2012a); [15]Perdiguero *et al.* (2012b); [16]Prunier *et al.* (2011); [17]Velasco-Conde *et al.* (2012); [18]Watkinson *et al.* (2003).

(Lorenz *et al.*, 2011; Perdiguero *et al.*, 2012b; Behringer *et al.*, 2015).

Genes involved in pathogen or biotic stress defenses are often upregulated during drought stress, but those involved in growth, including cell division and wall construction, are often downregulated (Dubos *et al.*, 2003; Lorenz *et al.*, 2006, 2011; Perdiguero *et al.*, 2012b; Behringer *et al.*, 2015) (Fig. 2; Table 1). Up or down changes in carbohydrate and lipid metabolism and protein handling pathways are also evident, although these are more difficult to interpret. Aquaporins, which affect membrane water permeability, were found to be upregulated in two studies (Lorenz *et al.*, 2011; Behringer *et al.*, 2015).

When drought-stressed seedlings are re-watered, most gene expression quickly returns to normal. In *Pinus taeda*, only 76 of the 2445 genes with altered expression during drought were still different after 48 h of recovery (Lorenz *et al.*, 2011). Lorenz *et al.* (2006) found 11 genes upregulated in 'recovered' *P. taeda* seedlings relative to either drought-stressed or well-watered seedlings, including probable cell wall proteins, an aquaporin and a gene involved in vacuole function. These may reflect recovery or repair processes that occur once drought stress is removed.

Studies targeting specific gene families or functions Gene families illustrate the complexity of expression responses to drought. *Pinus pinaster* has at least eight dehydrin genes, based on expressed sequence tag (EST) analyses (Perdiguero *et al.*, 2012a). Three of five were downregulated during drought, whereas the other two were upregulated (Velasco-Conde *et al.*, 2012). Most dehydrin induction occurred after 20 d of drought (Perdiguero *et al.*, 2012a), which may be why a similar but shorter study (Perdiguero *et al.*, 2012b) did not reveal the upregulation of dehydrins. Expression can also vary by tissue. Of seven dehydrins examined in *P. abies*, drought stress upregulated four in needles, but only two in bark, with one being downregulated in bark (Eldhuset *et al.*, 2013).

To investigate the link between drought and defense gene expression, Fossdal *et al.* (2007) exposed *P. abies* seedlings to a pathogen (*Rhizoctonia*), drought stress or both, and examined the transcription of 14 candidate defense genes. Genes were upregulated more slowly in drought-stressed seedlings than in pathogen-inoculated seedlings. The combined treatment led to more rapid and/or higher expression of many defense genes than either alone, which may be related to the synergistic mortality risks posed by biotic and abiotic stressors. Pleiotropic effects for some drought/defense-related genes are also possible, but none have been identified to date.

2. Local adaptation to drought in conifers

Provenance studies Multiple provenance studies have identified patterns consistent with local adaptation to drought. Trees from drier climates often exhibit conservative growth strategies (Fig. 2), such as slower height or needle growth (de la Mata *et al.*, 2014), less aboveground biomass or a shorter growing season (Kerr *et al.*, 2015). Seedlings from dry environments often also exhibit more root growth (Fig. 2) and higher drought survival (Cregg & Zhang, 2001; Matías *et al.*, 2014; Kolb *et al.*, 2016). Provenance trials of *Pinus halepensis* have shown mixed responses, with low growth and high water use efficiency (WUE) in dry-sourced populations (Voltas *et al.*, 2008), but high growth in populations from intermediate-aridity areas (Klein *et al.*, 2013), which may be related to growth plasticity.

Because of the importance of carbon resources for plants, WUE – the ratio of carbon fixed to water lost – has long been considered to be closely tied to drought tolerance (Farquhar *et al.*, 1989; Cregg & Zhang, 2001). Measures that integrate over longer time periods, such as the carbon isotope ratio $\delta^{13}C$ (Farquhar *et al.*, 1989; Livingston *et al.*, 1999), are most frequently used to represent changes in WUE in trees. However, although different measures of WUE are often correlated (de Miguel *et al.*, 2014), they are not interchangeable. For example, carbon discrimination (Δ) is sensitive to chloroplast carbon concentrations and mesophyll conductance, whereas WUE itself is heavily influenced by evaporative demand, which does not directly affect Δ (Seibt *et al.*, 2008). In addition, nitrogen fertilization can increase WUE and decrease Δ, but does not affect g_s or transpiration (Ripullone *et al.*, 2004). Thus, WUE and Δ do not always co-vary, and caution is needed in the interpretation of $\delta^{13}C$ as a measure of WUE. Additional caution is warranted when using WUE as an indication of drought tolerance. High WUE may not be adaptive in some dry environments if the use of less water per unit carbon fixed does not result in slower depletion of soil water (as a result of competition or other factors), or if plants with higher WUE grow faster and thus use more total water.

Although a few studies have shown higher $\delta^{13}C$ for populations from dry sites (Kerr *et al.*, 2015), others have shown the opposite (Guy & Holowachuk, 2001; Lamy *et al.*, 2011). There was no difference between three populations of *Pinus ponderosa* seedlings from varying climates in $\delta^{13}C$ or instantaneous WUE; the drought-adapted populations exhibited greater plasticity in water use (Zhang *et al.*, 1997). In *P. halepensis*, however, individuals from more mesic sources showed higher plasticity of WUE than those from drier sources (Klein *et al.*, 2013), but dry sources may show higher average WUE (Voltas *et al.*, 2008). Highly plastic growth and water usage reduce apparent WUE over the whole season compared with consistently moderate to low water usage (Zhang *et al.*, 1997; Kerr *et al.*, 2015).

Instantaneous measures of WUE can change over a day, whereas integrated measures can differ significantly for a source population grown under different conditions or for the same tree across years (Zhang *et al.*, 1997; Klein *et al.*, 2013). Changes in WUE may thus be a useful indication of drought stress, but, in conifers, radial growth and WUE are often weakly or negatively correlated (Adams & Kolb, 2004; Correia *et al.*, 2008; Eilmann *et al.*, 2010; Marguerit *et al.*, 2014). In pines, higher WUE usually results from reduced g_s (Fig. 2) and/or reduced leaf area (Zhang *et al.*, 1997; de Miguel *et al.*, 2012, 2014; Marguerit *et al.*, 2014; Reinhardt *et al.*, 2015), which can limit photosynthesis and growth (Brendel *et al.*, 2002). Low g_s can also result in higher tissue temperatures, which can be damaging, particularly in seedlings (Kolb & Robberecht, 1996).

Drought length and severity can influence measures of relative drought tolerance between populations. In *P. ponderosa* seedlings,

the relative growth rate under moist conditions was positively correlated with previously measured tolerance to severe drought, whereas, under 4-wk drought, the intermediate-drought-tolerant population grew faster (Zhang *et al.*, 1997). When Silim *et al.* (2001) examined *Picea sitchensis*, *P. glauca* and their hybrids, they found that *P. sitchensis* and the hybrids had the highest WUE and growth in well-watered conditions, but *P. glauca* and the hybrids had higher WUE and growth in drought conditions. Similarly, the relative transpiration and photosynthetic rates, WUE and growth of *P. halepensis* tree provenances differed between near-desert and Mediterranean planting sites (Atzmon *et al.*, 2004). Such shifts in ranking are often a result of plasticity differences between populations.

Provenances of *P. pinaster* from across the species' range did not vary in cavitation resistance, suggesting uniform selection or lack of genetic variation (Lamy *et al.*, 2011). In *P. halepensis*, however, the percentage loss of conductivity (attributed to cavitation) differed significantly between provenances, but not between environments (Klein *et al.*, 2013). Although plasticity has been observed in xylem wall thickening, time to thickening and number of cells in *Picea mariana* in drought experiments (Balducci *et al.*, 2013), cell anatomy studies often focus on only one population, so that the extent of local adaptation is unknown.

Genetic signals of local adaptation Genome scans have identified loci in conifers that may be under differential selection across environments (Fig. 2; Table 1). Of 13 candidate genes for drought response in *P. pinaster*, two showed signs of divergent selection, although only one (a cell wall gene) exhibited a pattern correlated with a climatic gradient; three, including two dehydrins, showed evidence of balancing selection (Eveno *et al.*, 2008). Prunier *et al.* (2011) examined SNPs from 313 candidate genes in *P. mariana* and found 16 that exhibited differentiation correlated with precipitation, including a LEA protein and genes in the ubiquitin protein handling pathway. However, differentiation between populations can be driven by processes unrelated to climatic gradients. Conifer populations are likely to violate the assumptions of such tests because they rarely form discrete isolated populations and are often far from demographic equilibrium; mis-specification of population hierarchical structure can lead to high false positive rates (Eckert *et al.*, 2010b). However, newer methods are being developed that avoid frequently violated assumptions and reduce false positives (Whitlock & Lotterhos, 2015).

The approach preferred by recent studies is to directly assess the association of loci with environmental gradients (G2E), whilst controlling for population structure (Fig. 2; Table 1). Jaramillo-Correa *et al.* (2015) examined the correlation of *P. pinaster* candidate gene SNP allele frequencies with climate principal component (PC) axes, using transcriptome-wide SNPs to control for population structure and demographic history. They identified 18 environmentally associated SNPs, many of which were in genes relating to carbohydrate transport, cell wall construction and photosynthesis. Two surveys of *P. taeda* examined associations between candidate gene SNPs and environmental gradients. One examined the association of these loci with five climatic PC axes (Eckert *et al.*, 2010a), whereas the other used an aridity index (precipitation/PET) for each county (Eckert *et al.*, 2010b). There was some overlap in function between the loci identified (Table 1). However, the studies disagreed on whether SNPs associated with climate also tended to be F_{st} outliers.

G2E associations have been detected even over short geographical distances, suggesting that selection can drive local adaptation in the presence of high gene flow. Eckert *et al.* (2015) examined *Pinus lambertiana* populations around Lake Tahoe, an area of *c.* 35×65 km^2, and found 11 genes associated with environmental PCs reflecting differences in water availability. These included genes involved in carbohydrate metabolism and transport and response to biotic stress (Table 1).

A cross-species comparison of environmental associations suggests some similarities in the genetic mechanisms involved in climatic tolerances across conifer genera. For each of four European conifer species in the Italian Alps, 6–18 SNPs (of 249–693 investigated) were associated with precipitation/temperature PC axes (Table 1). There was some overlap between species in the genes represented, including heat shock proteins, and cell wall construction and carbohydrate metabolism genes (Mosca *et al.*, 2012).

3. Linking genes to traits and traits to drought adaptation by combining approaches

Gene expression studies have identified a range of genes that may be involved in drought responses, but these results are not easily connected to the results of physiological or provenance response studies. First, RNA transcripts reflect the genes being expressed at a particular instant, whereas morphological or physiological traits are the result of processes acting over a longer time. Second, most gene expression studies do not examine differences between populations. Although some evidence suggests that stronger gene expression changes during stress are associated with greater growth or survival, different genotypes and demographic stages can show significant differences in gene expression changes (Hamanishi & Campbell, 2011).

A few studies have begun to address this. Provenances of *P. pinaster* differed in the expression response of two dehydrin genes, as well as in physiology and mortality rates (Velasco-Conde *et al.*, 2012). Similarly, three genotypes of *P. taeda* differed in their gene expression responses to drought and re-watering (Lorenz *et al.*, 2006). More such studies are needed, but care must be taken to distinguish between drivers of expression differences. For instance, a more drought-sensitive tree might express higher levels of dehydrins at a given drought stage because the leaf water potential has dropped faster than in a drought-resistant tree, whereas the resistant tree might express higher levels of dehydrins than the sensitive tree at a given leaf water potential.

Genome scan and G2E association studies can be useful tools in the search for genes responsible for local adaptation. Although such studies can identify loci at which allele frequencies differ between environments, it is not always clear how these differences are connected to phenotypic differences, and thus what traits are under selection in a given environment. This is where QTL and G2P association studies are useful.

Most conifer QTL studies have focused on wood traits, growth or yield. Of the two that have examined drought tolerance, the first identified four significant and four suggestive QTLs for $\delta^{13}C$ in *P. pinaster*, none of which co-located with QTLs for ring width (Brendel *et al.*, 2002). The second examined a wider range of traits – photosynthesis (A_n), chlorophyll fluorescence, g_s, $\delta^{13}C$, intrinsic WUE (WUE_i) and specific leaf area (SLA) – in F_1 cross seedlings of *P. pinaster* when well watered or after 1 or 2 wk without water, and identified 28 significant and 27 suggestive QTLs (de Miguel *et al.*, 2014). Locations of the QTLs for each trait (except SLA) varied by time point. Candidate genes within the QTLs were identified (Table 1): those for g_s and WUE_i included stomatal regulation, ABA signaling and cell wall construction genes; those for $\delta^{13}C$ included an aquaporin; and those for chlorophyll fluorescence included transcription factors and a histone chaperone.

G2P studies focusing on quantitative traits (including cold hardiness, budset date, yield and wood properties) have generally been successful in identifying associated loci (Holliday *et al.*, 2010; Resende *et al.*, 2012; Gamal El-Dien *et al.*, 2015). However, only a few studies have investigated drought tolerance in conifers (Fig. 2; Table 1), with less success. All such studies used $\delta^{13}C$ as the focal trait. As we argue in Section VI, other traits would probably yield results that are more helpful for the understanding of drought responses. Gonzalez-Martinez *et al.* (2008) examined 41 candidate stress response genes of *P. taeda*, using 61 tree families planted at two sites. However, drought stress was probably mild, and they only identified one strongly associated gene and one weakly associated gene at each site. A later study on the same species examining 3938 SNPs identified seven new associations with $\delta^{13}C$ (Cumbie *et al.*, 2011). Four of the associations were with unknown proteins, with only a transcription factor probably involved in the ABA-mediated stress response having an obvious connection to drought responses.

G2P and G2E association studies complement one another, with the first identifying loci linked to targeted traits, but not whether these loci are under selection in nature, and the second doing the opposite. The combination of these approaches is useful for the identification of genes and traits under selection in natural settings, but so far few studies have taken this approach. Eckert *et al.* (2015) tested the association of SNPs with five phenotypic traits and 11 environmental variables across 10 *P. lambertiana* populations around Lake Tahoe. This study identified six genes associated with phenotypic traits (bud flush date, needle nitrogen, $\delta^{13}C$ and height growth), and 31 associated with environmental PCs. Two genes were associated with both a trait and an environmental axis, including a glucose transport protein associated with $\delta^{13}C$ and environmental variables linked to water availability. A study focusing on multiple drought response traits and a larger number of SNPs might be able to identify more genes that have variants associated with both environmental gradients and drought tolerance traits.

Some traits and processes involved in drought response have been better studied at the genetic level than others (Fig. 2). Provenance studies have indicated that differences in stomatal control and shoot growth (physiologically related to all three drought response stages) are often involved in local adaptation to drought, and all other study types have identified the genes likely to be involved (related to the ABA pathway and cell division, respectively). However, although root growth has also been identified as important by provenance studies, root-growth-related genes have not been identified. Conversely, although genes related to resistance traits, such as changes in carbohydrate metabolism, and protective and pathogen defense molecule production, have been identified in expression or association studies, these traits have been largely ignored in provenance studies. Finally, xylem traits, including refilling ability, have not been the focus of any genetic study type.

V. Implications for the management of forests in a changing world

Tree improvement programs that aim to increase growth potential and stress resistance face the challenges of long generation times, the need for large-scale field experiments and the late expression of traits such as wood density (Isik, 2014; Gamal El-Dien *et al.*, 2015). Genomic selection, already routinely used in livestock breeding, has been proposed as a method of speeding up this process by using marker-predicted breeding values for phenotypes of interest (Grattapaglia & Resende, 2011; Isik, 2014). This approach is suitable for species with low LD and for traits with complex genetic architectures as it uses thousands of markers with effects that are estimated simultaneously (Gamal El-Dien *et al.*, 2015). As with traditional phenotypic selection, accuracy is likely to be greatest when tests are carried out in environments similar to the target environment, because of the high likelihood of genotype × environment interactions (Isik, 2014).

Several recent studies have demonstrated the potential of genomic selection approaches for traits of interest to forestry. Resende *et al.* (2012) carried out an early evaluation of genomic selection in *P. taeda*, making use of clonally replicated individuals grown on four sites and genotyped at 4825 SNPs. They found that the accuracy of prediction models within sites ranged from 0.63 to 0.75 for diameter and height, and estimated that the breeding cycle could be speeded up by 50% with this method. Gamal El-Dien *et al.* (2015) used GBS to genotype over 1000 interior spruce trees (*P. glauca* × *P. engelmanii*) over three sites that had been phenotyped for yield and wood attributes, and found that the incorporation of genomic information produced more accurate heritability estimates. Genomic estimated breeding values were most accurate (0.47–0.77) when data from multiple sites were used to fit the model.

Of even more relevance to selection for drought tolerance, Jaramillo-Correa *et al.* (2015) identified 18 SNPs associated with climatic PC axes in *P. pinaster*, and found that the frequency of locally advantageous alleles at these loci correlated with population-level survival rates in a common garden at the hot/dry end of the species range. Together with the growth trait analyses, these results suggest that association techniques could be applied to predict breeding values for overall drought tolerance or particular drought tolerance traits even though only some of the loci involved have been identified.

There is evidence of significant potential for selection approaches to improve drought responses in conifers. Provenance studies have

shown evidence of genetic differentiation between populations in drought responses, and genome scan and G2E associations are finding evidence of natural selection on within-species genetic variation. Second, heritabilities for drought tolerance traits, when these have been examined, tend to be moderate to high. The calculation of heritability requires pedigree information: parent–offspring or sibling and half-sib comparisons. Narrow-sense heritability is the fraction of the variance in a trait attributable to additive genetic variation, as opposed to environmental and non-additive genetic variation. Because heritability depends on both genetic variation in the population assessed and the degree of variation caused by the environment, estimates are not transferable between situations. In *P. pinaster*, estimates of $\delta^{13}C$ narrow-sense heritability ranged from 0.17 to 0.41, depending on how many individuals of what populations were assessed in what sites; and ring width (0.19–0.25) and height growth rates (0.35) were also moderately heritable (Brendel *et al.*, 2002; Lamy *et al.*, 2011; Marguerit *et al.*, 2014). In the same species, heritability of P_{50} (a measure of cavitation resistance) was 0.44, but this was driven more strongly by low levels of other sources of variation rather than high additive genetic variation (Lamy *et al.*, 2011). Across species, measured heritabilities for $\delta^{13}C$ range from the very high 0.7 for *Araucaria cunninghamii* to < 0.1 for *P. taeda* (Johnsen *et al.*, 1999; Prasolova *et al.*, 2001; Baltunis *et al.*, 2008).

Managers of wild forests are often focused on ensuring the resilience and function of the ecosystem rather than productivity. G2E and G2P association studies may help to identify seed sources that could be 'preadapted' to projected conditions for replanting in wildlands. However, wild trees face a range of challenges, including disease and competition, as well as drought (Sthultz *et al.*, 2009; Grady *et al.*, 2015). Stand structure (McDowell *et al.*, 2006; Das *et al.*, 2008, 2011; Fernandez *et al.*, 2012) and soil properties (Koepke *et al.*, 2010; Phillips *et al.*, 2016) may also directly affect how trees experience drought stress. Studies that integrate stand-level processes with genetic testing can further bridge gaps between genetic experiments and forest-scale management. Restoration projects could be used as experiments (Howe & Martínez-Garza, 2014) to test genomic predictions of survival and growth in a given environment, as well as the effects of genetic composition and diversity of the planted population on restoration success.

VI. Future directions

In order to address the remaining questions relating to the genetics of drought tolerance in conifers, we offer several recommendations for future studies.

1. More 'crossover' between the major categories of drought genetics studies

Common garden, gene expression and genetic association studies all have different strengths and weaknesses, and none alone will answer the question of how genetic differences affect drought tolerance (Fig. 3). As described previously, a combination of different types of association study may help to identify loci that are under selection in the wild and the traits they influence. Similarly, gene expression studies could easily be combined with common garden studies of adults or seedlings to address whether differences in drought responses between populations or genotypes are a result of differences in gene sequences, gene expression patterns or both.

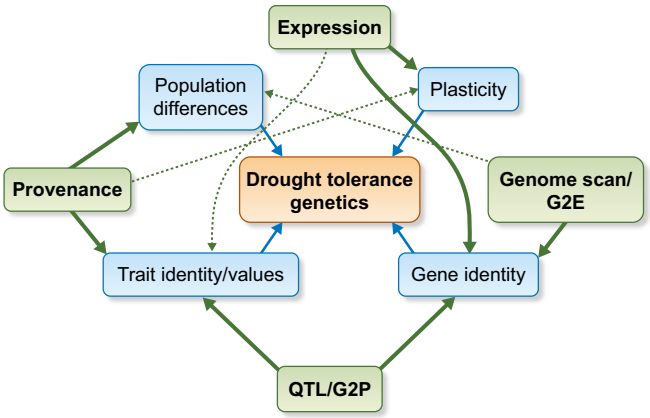

Fig. 3 The study types used to investigate drought tolerance genetics to date (green boxes) each address only some of the components (blue boxes) needed to understand the links between genes, phenotypes and fitness in a given environment. Some components are always addressed (solid line arrows), whereas others are sometimes addressed (dashed line arrows). A combination of study types can allow a more complete set of connections to be made: for instance, a combination of provenance and expression approaches can be used to investigate the role of expression differences in local adaptation. G2E, genotype-to-environment; G2P, genotype-to-phenotype; QTL, quantitative trait locus.

2. Less focus on WUE

Many studies to date have focused on WUE, often using $\delta^{13}C$ as a proxy. As discussed above, however, WUE is a ratio of changes in photosynthesis and transpiration, which can both vary, and higher WUE may or may not be associated with greater survival or growth in dry conditions. Moreover, different measures of WUE are not entirely consistent. We therefore recommend that future studies use survival and/or growth during and following drought as the metric of overall 'drought tolerance', and measure photosynthesis and water loss separately if these are processes of interest. The time involved in the measurement of traits for hundreds or thousands of individuals has encouraged the focus on easily measured $\delta^{13}C$, but much progress has been made in high-throughput phenotyping techniques (Plomion *et al.*, 2016). For instance, thermal and long-wave infrared sensors can measure leaf temperature or stomatal conductance, near and short-wave infrared sensors can measure leaf water content, and fluorescence sensors can measure chlorophyll content and photosystem efficiency (Araus & Cairns, 2014; Fahlgren *et al.*, 2015).

3. More focus on carbohydrate metabolism, xylem refilling and root growth

There are several traits and processes that have been suggested to be important for drought response by physiological studies, but about which there is little genetic information (Fig. 2). Genetic studies

frequently identify genes related to carbohydrate metabolism and transport as having altered expression or allele frequencies depending on water availability. It is difficult to make sense of these patterns because the link between these metabolic changes and tree function and survival during drought is still unclear. We also know relatively little about which species can refill cavitated xylem, under what circumstances and by what mechanisms. Thus, it is difficult to determine whether any genes identified by expression or G2E studies are involved in this process. Similarly, how roots and root growth respond to changes in water availability, and what genes are involved in these responses, remain poorly understood. Although the measurement of root architecture can be complex, high-throughput methods are being developed for this as well (Araus & Cairns, 2014).

4. More experiments using adult trees and longer timespans

Most experimental studies, including those looking at gene expression, have focused on seedlings. There may be important differences in how different life stages respond to drought. For instance, *Pinus nigra* ssp. *laricio* adults have been observed to follow an isohydric strategy, whereas seedlings in a glasshouse experiment did not (Cinnirella *et al.*, 2002). Although it is more complicated to impose drought treatments on adults, drought experiments have been carried out on adult trees using networks of rain shields/gutters to intercept precipitation and direct it away from the trees (Borghetti *et al.*, 1998; Cinnirella *et al.*, 2002; Plaut *et al.*, 2013). This water can also be re-directed to other plots to create 'well-watered' treatments. For the most part, these studies have been carried out on natural populations. However, if they were coupled with provenance study plantings, one could test for population or genotypic differences in adult drought response. Likewise, apart from some long-term provenance studies (Atzmon *et al.*, 2004), most experiments span a few days to a few months. In order to investigate drought resilience and legacy effects, more multi-year studies are needed.

5. Explicit consideration of different types of drought in the context of natural environments

The length and intensity of drought can affect which trait combinations result in greater fitness. In Section IV.1, we mentioned the great diversity of methods used to induce or measure drought stress treatments in gene expression studies. The same diversity is found in G2P and provenance studies as well. There is a need to assess: (1) whether environmental treatments (e.g. watering frequency or soil moisture) roughly match the range of conditions in the environments in which the target species does or might grow; (2) how environmental treatments relate to plant stress measures (e.g. leaf water content or wilting); and (3) whether traits, responses or genotypes associated with drought tolerance in the glasshouse or laboratory predict performance in the field. In addition, studies testing longer term drought treatments are lacking, as are those that explicitly test variable combinations of drought length and severity. Future work should address these gaps.

6. Identification of the role of 'mystery' genes

In most of the genetic studies cited above, a relatively high proportion of the genes expressed or linked to phenotypes or environmental gradients of interest either have unknown or poorly defined functions. Behringer *et al.* (2015), for instance, found that, of the 832 transcripts analyzed for gene ontology, 538 either had no database hits or could not be assigned to a biological process. Although this could be partly addressed with further studies in

Table 2 Frequency of inclusion of conifer taxa in drought tolerance and genomic studies, as well as features that influence their attractiveness for such studies

Conifer taxon	Representation in drought tolerance studies	Genomic information[1]	Aridity of natural habitat	Economic importance
Pinaceae				
Pinus	High, particularly *Pinus pinaster*	Full genome: *Pinus taeda*, *Pinus lambertiana*. Partial info for many	Mesic to xeric	Generally fast-growing. Plantations worldwide for pulp and wood
Picea	Moderate	Full genome: *Pinus abies* and *Pinus glauca*	Boreal areas (cold water limitation)	Cool climate plantations for pulp and wood
Pseudotsuga	Low	Full genome for *Pinus menziesii*	Mesic	Plantations for pulp and wood in Europe and North America
Other (e.g. *Larix*, *Abies*, *Tsuga*, *Cedrus*)	Extremely low	*Larix siberica* sequence in progress	Variable – *Cedrus* xeric, *Tsuga* mesic, etc.	Some high – *Larix* often planted, *Cedrus* wood historically valued
Cupressaceae	Low	Some candidate gene work in redwood, candidate genes and linkage maps in *Cryptomeria japonica*	Many mesic, but some Cuppressoideae (*Calocedrus*, *Cupressus*, *Juniperus*) inhabit xeric areas and many species anisohydric	Slow-growing. Only a few (e.g. redwood, *Sequoia sempervirens*) plantation grown for timber
Other families (e.g. Araucariaceae, Podocarpaceae)	Extremely low	Little	Variable, but often mesic (some tropical)	Some high-value timbers, esp. Araucariacea. Infrequently planted for wood or pulp

[1]http://www.pinegenome.org/projects.php

traditional model organisms, such as *Arabidopsis*, analysis of loblolly pine and Norway and Sitka spruce genome sequences suggests that there could be thousands of conifer-specific gene families (De La Torre *et al.*, 2014). This shortcoming must be addressed by further development of model systems in conifers.

7. Inclusion of overlooked conifer taxa

Unsurprisingly, the conifer taxa that have received the most attention in terms of drought tolerance studies or genomic studies are those that are of high economic value, especially those that are frequently grown in plantations. Species from xeric environments also tend to be over-represented in drought tolerance studies. This means that most drought tolerance genetics studies have been carried out on pines (*Pinus*), with a modest representation of spruce (*Picea*) and Douglas-fir (*Pseudotsuga*) (Table 2). Although this focus is understandable, this means that a number of ecologically or economically significant taxa have been left out, notably the Cupressaceae (Brodribb *et al.*, 2014). Many *Juniperus* and New World *Cupressus* and *Calocedrus* species are impressively drought tolerant. In the well-studied pinyon–juniper woodlands of the American Southwest, anisohydric juniper tends to exhibit lower mortality than *Pinus edulis* during severe drought (McDowell *et al.*, 2008; Koepke *et al.*, 2010). However, not all pine species exhibit isohydric behavior (Cinnirella *et al.*, 2002). Thus, the genes involved in drought response could differ substantially between families, genera or species.

VII. Conclusion

Conifer responses to drought can involve a variety of morphological and physiological traits. Provenance studies, gene expression studies and genomic approaches all have different strengths and weaknesses, and have revealed different aspects of how conifers respond and adapt to drought stress. The genes and pathways that are commonly involved include those related to ABA signaling, carbohydrate metabolism, the production of proteins and other molecules that protect cells from changes in osmotic potential or oxidative stress, and defense against biotic threats that might take advantage of a drought-stressed plant. In some cases, the same genes appear to be involved in the responses of distantly related species. However, not all traits thought to be involved in drought tolerance have been investigated from a genetic perspective, and drought response strategies are likely to differ between conifer species, populations and life stages in ways that have not been fully explored. The impact of these responses on fitness in turn depends on the intensity and duration of the drought stress. Further progress will require the combination of insights from these disparate approaches. Comparisons across species, families and life stages will probably yield insights into which responses are most adaptive under different circumstances. Nevertheless, early attempts at predicting phenotypes from genotypes suggest that genetic tools may be able to aid managers to select appropriate planting stock in the near future, at least for the better studied species.

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