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Screening techniques and sources of resistance to abiotic stresses in cool-season food legumes

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Summary

The adaptability and productivity of cool-season food legumes (chickpea, faba bean, lentil, pea) are limited by major abiotic stresses including drought, heat, frost, chilling, waterlogging, salinity and mineral toxicities. The severity of these stresses is unpredictable in field experiments, so field trials are increasingly supplemented with controlled-environment testing and physiological screening. For drought testing, irrigation is used in dry fields and rain-out shelters in damp ones. Carbon isotope discrimination ($\Delta^{13}\text{C}$) is a well-established screen for drought tolerance in C3 cereal crops which is now being validated for use in grain legumes, but it is relatively expensive per sample and more economical methods include stomatal conductance and canopy temperature. Chickpea lines ICC4958 and FLIP87-59C and faba bean line ILB938 have demonstrated good drought tolerance parameters in different experiments. For frost tolerance, an efficient controlled-environment procedure involves exposing hardened pot-grown plants to sub-zero temperatures. Faba beans Cote d'Or and BPL4628 as well as lentil ILL5865 have demonstrated good freezing tolerance in such tests. Chilling-tolerance tests are more commonly conducted in the field and lentil line ILL1878 as well as derivatives of interspecific crosses between chickpea and its wild relatives have repeatedly shown good results. The timing of chilling is particularly important as temperatures which are not lethal to the plant can greatly disrupt fertilization of flowers. Salinity response can be determined using hydroponic methods with a sand or gravel substrate and rapid, efficient scoring is based on leaf symptoms. Many lines of chickpea, faba bean and lentil have shown good salinity tolerance in a single article but none has become a benchmark. Waterlogging tolerance can be evaluated using paired hydroponic systems, one oxygenated and the other de-oxygenated. The development of lysigenous cavities or aerenchyma in roots, common in warm-season legumes, is reported in pea and lentil but is not well established in chickpea or faba bean. Many stresses are associated with oxidative damage leading to changes in chlorophyll fluorescence, membrane stability and peroxidase levels. An additional factor relevant to the legumes is the response of the symbiotic nitrogen-fixing bacteria to the stress.

Introduction

Abiotic stresses affect plant productivity in almost all agricultural settings. Extremes of water availability, temperature or mineral supply may be transient, lasting hours or days, or chronic, lasting throughout the

growing season. The cool-season grain legumes (pea, lentil, chickpea, faba bean) may be exposed to low temperatures and excess water early in the growing season (especially if autumn-sown) and high temperatures and inadequate water at the end of the growing season. Mineral toxicities (e.g., chloride, sodium, or

boron) are often present in marginal agricultural lands which would most benefit from additional diversity of crops. This review discusses developments since 1994 in screening and breeding for drought, cold, heat, waterlogging and mineral toxicity in chickpea, faba bean, lentil and pea. Common themes include escape, avoidance and tolerance (Ludlow & Muchow, 1990; Wery et al., 1994; Subbarao et al., 1995). The literature since 1994, when the area was last reviewed in *Euphytica* in a special issue of volume 73, varies in depth and breadth, depending on the stress and the crop.

Over the past decade, there has been considerable progress at increasing the adaptation of chickpea (*Cicer arietinum* L.) and lentil (*Lens culinaris* Medik. ssp. *culinaris*) to autumn-sowing and there is a large body of literature on drought responses, particularly in chickpea and faba bean (*Vicia faba* L.). The literature on salinity tolerance is rather smaller and that on waterlogging is smaller again. The literature on the biochemical nature of the responses, some of which may be used as screening tools, has burgeoned, particularly in pea (*Pisum sativum* L.).

Field screening for stress response often involves growing germplasm lines in contrasting conditions and estimating a susceptibility index from the relative yield in the two environments, as initially proposed by Fischer and Maurer (1978) for drought purposes. A susceptible check is usually included at frequent intervals. The nitrogen-fixing bacteria can also be affected by the stress and maintenance of the symbiosis and is an important part of stress response in legumes. A key, recurring problem in screening for abiotic stress tolerance is environmental heterogeneity. Climatic stresses such as drought or frost may not occur in the year in which the trial is run or may be so severe that all accessions in the trial are killed. Soils vary in water retention capacity, compression, aeration and mineral content, in the space of a few centimetres. Thus, plants that appear to be tolerant or resistant to the stress have often escaped it instead. While land for testing genetic materials is often cheap, in-field modifications such as homogenised soil beds or rain-out shelters are limited in size and are expensive. Furthermore, field trials generally run the whole growing season. Therefore, it is often considered desirable to have a controlled-environment screening system, where the response may be evaluated uniformly and rapidly. A screening system may become generally acceptable when it is based on simple selection criteria, provides rapid and accurate screening of large numbers, is non-destructive, reproducible and relates to field performance (Saxena et al., 1994; Wery

et al., 1994; Serraj et al., 2003). At the next level of resolution, physiological parameters have been investigated in relation to their suitability as screening techniques for abiotic-stress tolerance. These measure an instantaneous response instead of requiring integration of the whole season into the yield. Eventually, appropriate physiological tests can be used in the field either as a preliminary mass-screening method or as a definitive, environmentally robust screen. Sources of resistance identified in these ways can be crossed in order to pyramid complementary genes for the same or different response mechanisms. Genetic populations created in this process can be used for mapping quantitative trait loci (QTL) and then developing marker-assisted selection (MAS). Finally, intensive experimentation on model species such as *Arabidopsis thaliana* and, increasingly, the model pasture legume *Medicago truncatula*, is casting light on the overall pathways involved in various stress responses. These results may lead to technological interventions to increase stress resistance in the cool-season grain legumes.

Drought stress

Drought is considered the major abiotic stress in many parts of the world (Johansen et al., 1994; Malhotra et al., 2004) and is responsible for heavy production losses in cool-season food legumes (Saxena, 1993; Singh et al., 1994; Subbarao et al., 1995). Some losses are due to intermittent drought during the vegetative phase while others are due to terminal drought during reproductive development (Erskine et al., 1994; Singh et al., 1997a; Serraj et al., 2004b). The severity of drought stress is unpredictable as it depends on many factors such as occurrence and distribution of rainfall, evaporative demands of the atmosphere and moisture storing capacity of the soils (Wery et al., 1994). Therefore, characterization of the drought pattern of the target environment is an important step in designing strategies to alleviate drought stress (Subbarao et al., 1995).

Drought escape occurs when phenological development is successfully matched with periods of soil moisture availability, where growing season is short and terminal drought stress predominates (Turner, 1986). Therefore, the success of increasing legume production in drier regions prone to terminal drought largely depends on the development of short season varieties that enable the crop to escape severe soil-water deficits (Erskine et al., 1994; Siddique et al., 2001; Berger et al., 2003). Avoidance is related to the maintenance of

high tissue water potential and consists of mechanisms that both reduce water loss from plants, due to stomatal control of transpiration, and also maintain water uptake, through an extensive and prolific root system (Passioura, 1987). Dehydration tolerance refers to the ability of the plants to withstand low tissue water potential (Turner, 1986). The effect of internal water status on plant functions provides clues concerning mechanisms of drought tolerance.

Heat stress

Heat stress is most often associated with drought stress and is particularly detrimental to grain legumes because it affects crops directly by impairing physiological process and indirectly by affecting plant–water relations (Paulsen, 1994; McDonald & Paulsen, 1997). The effect of high temperature on growth is complex because plant responses are influenced by various factors such as the diurnal temperature range and water stress (Paulsen, 1994). Thus, the complete understanding of high temperature tolerance of plants require a thorough investigation of various physiological processes. Drought and high temperatures occur together in many environments important for legume production, and both stresses can interact to reduce yields (McDonald & Paulsen, 1997). Often these stresses coincide with the phase of reproductive development in legumes (Ali et al., 1994). McDonald and Paulsen (1997) found that faba bean was more sensitive to soil-moisture deficit than pea, wilting at higher water potentials. They proposed a linkage between the sensitivities to drought and heat stresses and suggested that selection for one tolerance may improve the other.

Field screening techniques

Singh et al. (1997a) considered that the lack of efficient screening techniques impeded efforts to breed for drought resistance in food legumes. The two main methods for controlling water supply are line-source sprinkler irrigation, which provides a gradient of water deficits (Hanks et al., 1976) and rainout shelters (Serraj et al., 2003). These systems have been used to identify sources of resistance or tolerance to drought stress (Table 1). Link et al. (1999) and Abdelmula et al. (1999) assessed drought tolerance in faba bean as the ratio of yield under drought to yield under well-watered conditions, using a rainout shelter. Genotypes from North Africa and Latin America were generally more drought tolerant than European cultivars (Link

et al., 1999). Among the inbred lines, relative yield (droughted/non-droughted) varied from 53% in a line derived from the German cultivar Troy, to 83% in Giza-3, from Egypt (Abdelmula et al., 1999).

For rapid screening purposes, Singh et al. (1997a) proposed a 9-point scale for drought tolerance in chickpea, as commonly used for other environmental stresses. The optimum time for scoring was the late pod-filling stage, near maturity. Rating 1 indicated a plot free from effects of drought stress while rating 9 indicated no seed-set due to the severity of drought. With this method, 19 of 4165 chickpea lines were identified as drought resistant (Table 1) and have been used by ICARDA and other national breeding programmes.

Selection for drought resistance based solely on grain yield may not bring genetic improvement in the desired physiological traits and may not detect when different mechanisms have similar outcomes (Subbarao et al., 1995). In the last decade, appreciable progress has been made in examining the various physiological parameters associated with drought response.

Leaf–water relations

Decrease in relative water content (RWC) was a main factor resulting in reduced growth in response to osmotic stress in pea (Alexieva et al., 2001). Under drought stress, sensitive pea genotypes were more affected by the decline in RWC than tolerant ones (Upreti et al., 2000). Large differences in midday leaf water potential were observed in rainfed legume crops, with chickpea and lentil reaching -3.3 MPa while pea and faba bean reached -2.0 MPa, but only chickpea maintained photosynthesis below -3.0 MPa (Leport et al., 2003). In faba beans, determination of leaf water potential was useful in describing the simulated drought stress, but was not very suitable for discriminating tolerant genotypes (Riccardi et al., 2001), suggesting that water potential was not the defining feature of the tolerance. Water retention in the morning and water content in leaves in the afternoon were potentially useful for screening drought tolerance in chickpea (Pannu et al., 1993).

Manzanares et al. (1998) found that those physiological parameters that were related to decrease in canopy temperature were beneficial to seed yield in pea under drought. Epicuticular wax content and leaf colour were of special interest as they reduced canopy temperature without water loss. Gupta et al. (2000) found that drought susceptibility index was significantly lower

Table 1. Identified sources of resistance or tolerance to drought and heat stress

Species	Accession	Selection criteria	Reference
Chickpea	FLIP 87-59C	Stress yield	Singh et al., 1996
Chickpea	FLIP 92-154C	Stress yield	Toker and Cagiran, 1998
Chickpea	RSG-44	Drought susceptibility index	Gupta et al., 2000
	RSG-143-1	Drought susceptibility index	
	ICC 4958	Membrane injury	
	ICC 4958	Seedling growth	
Chickpea	FLIP 92-60C	Osmotic regulation	Farshadfar et al., 2001
		Mean productivity	
		Stress tolerance index	
Chickpea	ICC 4958	Root dry mass	Krishnamurthy et al., 2003
	ICC 5337		
	ICC 7255		
	ICC 8261		
	ICC 13077		
	ICC 15294		
	K 850		
	K 850		
Chickpea	ILC 142	Drought resistance score	Singh et al., 1997a
	ILC 588	Drought susceptibility index	
	ILC 1306		
	ILC 2516		
	ILC 3550		
	ILC 3764		
	ILC 3832		
	ILC 3843		
	FLIP 87-7C		
	FLIP 87-8C		
	FLIP 87-59C		
	FLIP 88-42C		
	FLIP 88-42C		
Faba bean	Bourdon	Drought susceptibility index	Grzesiak et al., 1996a
	Gobo		
	Nadwislanski		
Faba bean	ILB 938	Stomatal conductance, Carbon isotope discrimination	Khan and Stoddard, unpublished
Lentil	MI-30	Leaf water traits	Salam and Islam, 1994
Pea	Mike, Solara	Drought susceptibility index	Grzesiak et al., 1996a
Pea	Arka Ajit	Relative water content	Upreti and Murti, 1999, 2000
		Sap osmotic potential	
		Stress yield	
Pea	Acc.623	Temperature induction response	Srikanthbabu et al., 2002
	Acc.765		

in chickpea lines RSG-44, RSG-143-1 and ICC 4958 (Table 1). The tolerant cultivars also exhibited lower membrane injury and higher seedling growth, osmotic regulation and water-use efficiency. Salam and Islam (1994) identified lentil mutant line MI-30 as high yielding at limited water supply as its leaf-water attributes allowed it to avoid drought.

Osmotic adjustment

Osmotic adjustment is a specific response to maintain water relations (turgor) under osmotic stress. A range of so-called osmotically active or compatible substances is involved, including soluble sugars, sugar alcohols, proline, Ca^{2+} , K^+ , and Cl^- .

Osmotic adaptation was found to be low or even absent in faba beans (Amede et al., 1999; Sau & Minguez, 2000; Katerji et al., 2002; Amede & Schubert, 2003a), moderate in pea (Yan et al., 1994; Amede & Schubert, 2003a) and high in both chickpea (Leport et al., 1998; Amede & Schubert, 2003b) and lentil (Leport et al., 1998; Leport et al., 2003). Drought tolerant pea genotypes had better turgor maintenance, which was significantly related to osmotic adjustment (Sánchez et al., 1998). Chickpea genotypes tolerant to drought in terms of drought susceptibility index had higher values for osmotic adaptation and water-use efficiency (Gupta et al., 2000), whereas biomass and grain yields of cool-season legumes did not correlate well with osmotic adjustment under rainfed conditions (Turner et al., 1996).

In chickpea, sugar and sugar alcohols contributed more than 50% (Amede & Schubert, 2003a), in peas from less than 10% (Yan et al., 1994) up to 46% (Sánchez et al., 2004) to the osmotic pool. In chickpea, pinitol and sucrose accumulated in response to drought stress (Orthen et al., 1994). In 49 pea cultivars, the stimulation of sugar levels induced by drought was proportional to osmotic adaptation and related to turgor maintenance (Sánchez et al., 1998). Cultivars that maintained turgor also exhibited higher harvest index. In several lines of faba bean, soluble sugar content in shoots and roots increased as the stress level increased, and only one tolerant line, 2/4, continued growth at 30% field capacity and accumulated soluble sugars in the root rather than in the shoot (El-Tayeb & Hassanein, 2000). Hence, that production of organic solutes during drought enhances the capacity of osmotic adjustment through maintenance of cell turgor and dehydration avoidance.

The accumulation of free proline has been found in many species under a range of abiotic stresses, mainly when osmotic stress is involved, although its mode of action in stress response is not clear at present (reviewed by Kuznetsov & Shevyakova, 1999). Proline, which is easily determined photometrically (Bates et al., 1973), is often considered as an osmoregulatory solute, but there is increasing evidence that it makes little contribution to osmotic adaptation in legumes (Shabala et al., 2000; Sánchez et al., 2004). Proline content increased under drought stress in pea (Sánchez et al., 1998; Alexieva et al., 2001), cultivars with high proline accumulation having lower water contents upon turgor loss. In faba beans, external proline application reduced membrane injury under drought stress (Gadallah, 1999) and genotypes with high proline accumulation had lower changes in the effective quantum

yield Φ_{PSII} of chlorophyll fluorescence (Balko, 2004). Proline accumulation was correlated with the drought susceptibility index, but not with yield in stressed faba beans (Balko, 2004).

Gas exchange

Stomatal closure is one of the first steps in adaptation to drought stress and it relates to dehydration avoidance by reducing water loss and maintaining water status during unfavourable conditions. Differences in stomatal resistance and leaf water status were shown when legume varieties were exposed to drought (Grzesiak et al., 1997) and water conservation was attributable to efficient stomatal regulation in legumes (Amede & Schubert, 2003b).

Carbon isotope composition has been used to study the genotypic and environmental responses of water-use efficiency of many crops (Martin et al., 1999; Condon et al., 2002). When stomata are open, photosynthesis in C3 plants discriminates against ^{13}C , which represents about 1% of the carbon in the biosphere, but when they are closed the plant can not discriminate. Carbon isotope discrimination ($\Delta^{13}\text{C}$), a measure of the $^{13}\text{C}/^{12}\text{C}$ ratio in the plant material relative to the value of the same ratio in the air where the plant grew (Farquhar & Richards, 1984), has been proposed as an indirect selection method for transpiration efficiency and water-use efficiency in several C3 species (Condon et al., 2002). The few studies on $\Delta^{13}\text{C}$ in food legumes show its value as a marker for water-use efficiency. Values of $\Delta^{13}\text{C}$ showed significant variation among lentil lines but not between water regimes (Matus et al., 1996). The ranking of genotypes for $\Delta^{13}\text{C}$ remained constant across various growth stages, so sampling could be done at any stage of plant development (Matus et al., 1995). Similarly, $\Delta^{13}\text{C}$ of lentil germplasm from 15 countries showed a highly significant, negative correlation with water-use efficiency, demonstrating the usefulness of this marker for efficient selection (Johnson et al., 1995). In field peas, better water-use efficiency of conventional tall cultivars compared to semi-dwarfs was shown by means of $\Delta^{13}\text{C}$ (Armstrong et al., 1994). Faba bean inbred lines of diverse genetic background exhibited considerable variation in $\Delta^{13}\text{C}$ which was negatively correlated with stomatal resistance and leaf temperature (Khan & Stoddard, unpublished data). The value of carbon isotope discrimination in screening is clear, but the cost per sample is high and other cheaper methods are sought. $\Delta^{13}\text{C}$ shows strong correlations with both stomatal conductance, which is

labour-intensive to measure, and canopy temperature, which is rapid and economical to measure (Khan and Stoddard, unpublished).

Nitric oxide (NO) plays a protective role against oxidative damage in many biological pathways. Garcia Mata and Lamattina (2001) investigated the effect of two NO donors, sodium nitroprusside (SNP) and *S*-nitroso-*N*-acetylpenicillamine, on faba bean in water-stress conditions. NO was able to induce 65% stomatal closure in faba bean, indicating that exogenous NO might increase tolerance to severe drought.

Under drought stress, the content of abscisic acid (ABA), which is associated with stomatal closure, increased in peas, more in the tolerant genotype than in the susceptible (Upreti & Murti, 1999). In a range of faba bean germplasm, however, ABA content did not correlate significantly with drought tolerance (Riccardi et al., 2001).

Root characteristics

Selection for deep and extensive root system has been advocated to increase productivity of food legumes under moisture-deficit conditions as it can optimize the capacity to acquire water (Subbarao et al., 1995; Serraj et al., 2004a; Sarker et al., 2005). Turner et al. (2001) identified rooting depth and density as a main drought avoidance trait in grain legumes for use in terminal-drought environments. Grzesiak et al. (1997) showed that drought resistant faba bean and pea cultivars had extensive and prolific root systems.

Chickpea genotype ICC 4958 was identified on the basis of its deep and extensive root system, as one of the most drought-resistant of more than 1500 diverse germplasm lines screened at ICRISAT (Saxena et al., 1993). Krishnamurthy et al. (2003) assessed genetic variation for root system traits under drought conditions in 211 accessions of chickpea and 10 accessions of its wild relatives, and compared it with 12 cultivars. Cultivars ICC 4958 and K 850 had the two highest values of root dry mass (Table 1). The total root dry mass also showed a significant linear relationship with total shoot dry matter and total leaf area of the plants. Serraj et al. (2004b) evaluated the root traits of 257 recombinant inbred lines (RILs) derived from a cross between ICC 4958 and cultivar Annegri to identify QTL for desirable root traits and to investigate relationship between root traits and seed yield under terminal drought stress. Considerable genetic variation was observed amongst the RIL population for root-length density and root dry mass at vegetative stage and in

a number of lines, a large root system was associated with higher seed yield.

Oxidative stress

Alterations in structure and function of cell membranes are early effects of stress injury and have been attributed to oxidative damage from active oxygen species. Membrane stability of plant tissues, mostly leaves, is often determined by electrolyte leakage measured as electrical conductivity. Chlorophyll fluorescence has also proved to be a relatively sensitive indicator of direct or indirect stress effects on photosynthesis (Schreiber & Bilger, 1993).

Membrane damage was not only found after exposure to drought stress in pea (Alexieva et al., 2001), but was also a suitable screening technique for drought-tolerance rating in legume species and cultivars (Grzesiak et al., 1996b; chickpea: Gupta et al., 2000; Deshmukh & Kushwaha, 2002). Heat stress decreased membrane stability in faba bean leaf discs (Hamada, 2001). Heritability of membrane damage response was low to intermediate in cowpea (Thiaw & Hall, 2004) and this needs investigation in the cool-season legumes.

Under conditions of drought, the electron transport chain was down-regulated as a response to stomatal closure, and therefore, substrate limitation in pea (Cornic & Fresneau, 2002). Both the effective quantum yield Φ_{PSII} , a measure of photon efficiency of linear electron transport of photosystem II (PSII), and the variable fluorescence $F_v = F_m - F_0$, (the difference between maximum fluorescence in the dark-adapted state and the minimum), a measure of the efficiency of open reaction centres of PSII translating photon energy, indicated drought stress response in pea leaves (Apostol et al., 2003). In faba beans, the decrease in Φ_{PSII} in leaves under drought stress was negatively correlated with both membrane stability and proline accumulation and positively related to yield stability (Balko, 2004).

Heat stress decreased the activity of PSII in pea leaves (Srivastava & Strasser, 1996). The effects started at 30 °C and were partially reversible (Briantais et al., 1996). At 40 °C, a sharp increase in F_0 together with a decrease in both F_v and the variable fluorescence ratio (F_v/F_m) indicated damage to the photosynthetic apparatus (Georgieva & Lichtenthaler, 1999). Inter- and intra-specific variation for membrane stability under heat stress was found in several legumes (chickpea as well as groundnut, pigeonpea and soybean) and the character was negatively correlated to F_v/F_m (Srinivasan et al.,

1996). According to the decrease in F_v/F_m , the ranking from heat tolerant to sensitive was in the order groundnut, soybean, pigeonpea and chickpea. Genotypic variation within species was found, and a test with detached leaves was considered to be suitable for screening (Srinivasan et al., 1996). Chlorophyll fluorescence appears to be a very promising tool to enable detection of stress-induced injuries as well as determination of stress tolerance (Methy et al., 1994).

Winter tolerance

Three phenological groups of cool-season food legumes are widely recognised: 'spring', 'Mediterranean' and 'winter'. In addition, care must be taken to distinguish chilling experiments, to temperatures below 15 °C at sowing or flowering, from those on freezing response during the vegetative growth phase.

Spring types are sown, as the name implies, in the spring, often into relatively cold soil and are seldom exposed to chilling conditions. Emergence from cold soil was poorer in white-flowered spring faba beans with low tannin content in the testa, than in normal-tannin isolines (Kantar et al., 1994).

Mediterranean types are sown in the autumn to benefit from high water-use efficiency in the cool, moist growing conditions of a relatively mild winter, where temperatures rarely fall below -8 °C, so there is no true winter dormancy phase. Thus, they avoid terminal drought and heat as well as developing a higher yield potential on larger plants suitable for mechanical harvest. While faba beans are very well adapted to Mediterranean winters, this growth pattern is relatively new in chickpea and lentil, so these crops are exposed to a new range of stresses.

Lentil production has been increased significantly by shifting sowing from spring to early spring or autumn-sowing in areas such as the highlands of Central Anatolia of Turkey, Iran, Afghanistan, and Balochistan province of Pakistan. The significantly higher biomass from the winter crop provides an additional benefit as the straw is a highly-valued animal feed. Similarly, the benefits of autumn-sowing of chickpea offers have been demonstrated at ICARDA, together with national partners in Algeria, Lebanon, Morocco, Syria, Tunisia, Turkey and Iran (Singh & Saxena, 1996; Singh et al., 1997b). Trials conducted for several years in Syria and Lebanon showed that winter-sowing could produce almost double the yield of traditional spring-sown chickpea, provided that

cold tolerance was adequate. In the Mediterranean region, approximately 200,000 ha of chickpea are now autumn-sown.

At higher latitudes, true-winter types require a dormant phase during which little growth is accumulated, combined with a much greater degree of frost tolerance than is required in the Mediterranean-type zone. Winter faba beans develop a short shoot with 2–3 leaves combined with a relatively high dry-matter content. Winter beans are mostly grown in U.K., where they have existed for at least 500 years, and coastal areas of France, and there is interest at expanding the range into regions with colder continental-type winters (Bond & Crofton, 1999; Link, work in progress). Winter beans have a considerable capacity for regrowth, tillering and healing of mechanical injury.

The recent literature on winter hardiness in peas is much smaller than in the other three species considered here. In Britain, winter peas are not at present very successful, due to inadequate winter hardiness, insufficient disease resistance and an unpredictable harvest date (Weightman, 2005). Early flowering in this material also increased the risk of exposure of the flowers to frost. Useful variation in the time to flowering was identified in a range of European winter pea materials (Lejeune-Henaut et al., 1999). The most important QTL for frost resistance co-segregated with the gene for delayed flowering (*Hr*), thus emphasizing the importance of appropriate flowering time for successful overwintering (Lejeune-Henaut et al., 2004).

Screening for survival

Following field tests of radiation-frost injury in a population of recombinant inbred lines of lentil, a single gene was identified conferring a highly significant degree of resistance (Eujayl et al., 1999). A RAPD marker co-segregated with this gene at a distance of 9.1 cM and may be used in marker-assisted selection. A controlled-environment test was developed for frost tolerance of lentil, involving hardening seedlings at 10 ° day/0 ° night for 1–6 weeks, then bringing them to -15 ° for 3 h (Ali et al., 1999). Under these conditions, narrow-sense heritability was very high and useful transgressive segregants were identified (Ali & Johnson, 2000). Similarly, heritability estimates for winter hardiness ranged up to 0.907 in a range of populations of recombinant inbred lines exposed to winterkill in several environments (Kahraman et al., 2004a). QTLs for winter hardiness have been identified in lentil and would be

useful for marker-assisted selection to enhance this trait (Kahraman et al., 2004b).

An important part of winter habit in chickpea is the sensitivity of the flowers to chilling. Temperatures below 15 °C cause damage, particularly to pollen tube growth in the style (Clarke & Siddique, 2004). There is genetic variation in chilling response, with some genotypes maintaining pod number even following a 5 °C event (Sandhu & ArasaKesary, 2003). In an innovative experiment, Clarke et al. (2004) applied pollen selection for chilling tolerance in chickpea. Pollen of the cold-tolerant line fertilized more ovules than pollen of the cold-sensitive line, when applied in equal numbers to chilled (12° day/7° night) flowers. Selection for pollen tube growth in chilled flowers was also applied to the segregating generations from the cross, leading to the release of two new cultivars which were also selected for Ascochyta disease resistance (Clarke et al., 2004). Winter vigour was identified as a key limiting factor for winter chickpeas in Australia (O'Toole et al., 2001).

Temperatures of 2–8 °C are effective for both hardening (inducing frost resistance) and vernalization; and full hardening can be achieved within 3 weeks under such conditions. European winter faba beans can tolerate temperatures down to –15.5 °C under controlled conditions (Herzog, 1989b). Some Japanese large-seeded cultivars tolerate long periods (>50 days) under snow cover (Fukuta & Yukawa, 1998). Across 4 years, winter survival of faba beans in Northern Germany varied between 0 and 100%, with most of the variation due to environment rather than to genotype (Herzog, 1989a). Duc & Petitjean (1995) used 3 weeks of hardening followed by 3 weeks of daily freeze-thaw cycles, with aggravating frost, from 0 °C to –6 °C. Injury was assessed as score of leaf damage on the basis of the methods of Herzog (1987) and the artificial test corroborated field data on overwintering. At present, Link and Arbaoui (unpublished) used artificial freezing of whole plants after 10 days of hardening to analyse genetic variation for frost resistance and to develop experimental lines. Provocative frost tests in pots expose roots to harder frost temperatures than when sown in the field (Figure 1).

Physiological and biochemical tests

Several physiological traits have shown promise for screening against cold stress response in other species (e.g., conductivity measurements of injured tissue, fatty acid composition of membrane lipids), but there are few

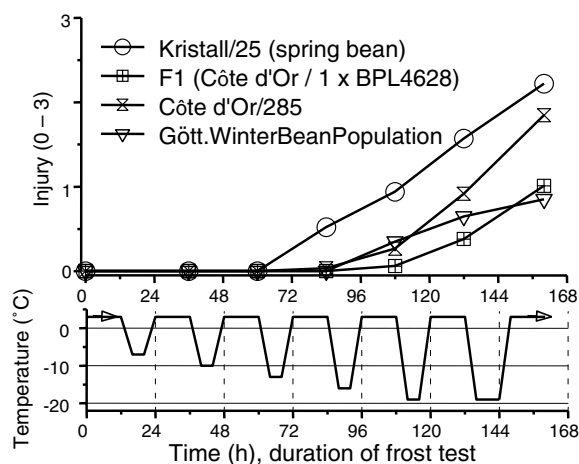


Figure 1. Result of artificial frost test with hardened, young faba bean plants in pots (visual score of frost injury, on the basis of discoloration and loss of turgor; 0 = no injury, 3 = max. injury).

reports on their use in grain legumes. From research in *Arabidopsis*, Thomashow (2001) presented strong support for the notion that a fundamental role of cold-inducible genes is to protect plant cells against cellular dehydration, with the CRT/DRE (C-repeat/dehydration responsive genetic element) imparting responsiveness to both chilling and dehydration. In hardened faba beans, the ratio F_v stressed/ F_v unstressed was found to be a measure of freezing resistance when compared with minimum temperature values from freezing tests (Herzog & Olszewski, 1998). Nevertheless, visual scoring was equally reliable and even showed some advantages (Olszewski, 1996). Chilling stress led to a decrease in chlorophyll fluorescence parameters F_v/F_m and Φ_{PSII} in soybeans (Guy et al., 1997), whereas F_v/F_m did not change significantly in the more chilling-tolerant pea (Georgieva & Yordanov, 1993). The ratio between the red and far-red fluorescence bands (F_{685}/F_{730} value) decreased in chilling-sensitive common bean and remained constant in chilling-tolerant pea, suggesting the suitability of this parameter as an indicator of plant chilling sensitivity (Agati et al., 1996).

Under chilling stress, pea leaves accumulated high quantities of sucrose, glucose-6-phosphate, fructose-6-phosphate and mannose-6-phosphate (Streb et al., 2003) and a close relationship was found between soluble sugar concentration of leaves just before the frost and the degree of freezing tolerance obtained by the different genotypes (Bourion et al., 2003).

Glycine betaine is, like proline, one of the compatible substances and there is a substantial literature on its role in stress response in many plant species.

There is, however, little evidence about cool-season legumes accumulating glycine betaine following osmotic stress, thus its possible role as screening marker is still to be investigated. During freeze-thaw cycles, exogenous glycine betaine prevented changes in relative proportion and antenna sizes of PSII in pea (Busheva and Apostolova, 1997). Exogenous ABA treatment improved freezing tolerance, but did not replace cold hardening in peas (Welbaum et al., 1997).

A relative damage index, calculated from membrane permeability, differentiated frost resistance of 19 pea cultivars, hybrids and lines (Voican et al., 1995).

Sources of resistance

The most prominent source of outstanding frost resistance in faba bean is the old French landrace Côte d'Or (Table 2), which survived -25°C without snow in 1984/85 in France (data from across four locations), whereas cv. Bourdon, generally considered robust, showed only 14% survival (Picard et al., 1985). A further, widely validated, source of frost resistance is ICARDA accession BPL 4628 from China (Duc & Petitjean, 1995) and several others are listed in Table 2. Highly-resistant accessions were sourced from many areas and the most promising were from mountainous regions in Central Asia, most probably because of the marked risk of frost early in the vegetative period (Olszewski, 1996). In Europe, large-seeded types are not included among the very winter-hardy material and such material is presently under development (D.A. Bond, personal communication, 2004).

In lentil, as in faba bean, sources of winter hardiness were associated with high-altitude locations and come from within the cultivated species, rather than from wild relatives (Hamdi et al., 1996). Varieties with winter-hardiness (such as Kafkas, Cifci and Uzbek) are now being cultivated in Turkey. Several additional lines were identified for future release (Table 2). In Iran, lines with winter-hardiness, early growth vigour and rapid ground cover are under on-farm evaluation for future release (Table 2). The Arid Zone Research Center, Balochistan, Pakistan released cultivar 'Shiraz-96', selected from ICARDA germplasm, for winter cultivation.

In chickpea, much vegetative-phase cold tolerance has been derived from interspecific crosses between the cultigen (*C. arietinum*) and wild relatives *C. reticulatum* and *C. echinospermum* (Singh et al., 1995; Table 2). Derivatives of such crosses gave nearly 3-fold increases in yield (Malhotra, 1998). Through gene

pyramiding, winter – hardiness was improved and cultivars Ghab-4 and Ghab-5 were released in Syria for winter cultivation.

Salinity and sodicity responses

Saline soils are defined by an electrical conductivity (EC) of $>4\text{ dS/m}$, which equates approximately to 40 mM NaCl. In addition to sodium and chloride, they often have elevated concentrations of sulphate and other ions and inadequate levels of others, such as calcium. Sodic soils are distinguished by a high bicarbonate content, associated with a high pH and an exchangeable sodium percentage (ESP) >15 , but not necessarily the high EC. Saline-sodic soils combine high EC with high ESP. In the 1994 review, salinity tolerance in faba beans was considered low, but variation was available (Bond et al., 1994); it was considered an important trait for chickpea (Singh et al., 1994) but not for lentil (Ersikine et al., 1994). Tolerance in faba bean exceeded that in chickpea, which was almost equivalent to lentil, while there was little information on pea (Saxena et al., 1994).

Screening methods

Field screening for salinity or sodicity tolerance is problematic as the distribution of ions, both vertically in the soil profile and horizontally across the field, is notoriously variable. Rainfall or dry weather at different times of the season add further confounding factors. An overhead sprinkler system was considered as effective as drip irrigation for discriminating genotypes and was much more economical to install (Isla et al., 1997). In another example, used for rice but adaptable for other crops, soil variability was handled by the excavation of large beds, filled with a defined soil mix and irrigated with a consistent water source (Mahmood et al., 2004). This option is realistic, however, only where labour is relatively inexpensive.

Crop responses to salinity depend on age, with some being more sensitive at germination, others at flowering, and still others at pod filling. Germination or early survival at high salinity ($>200\text{ mM NaCl}$) was considered not predictive of growth at more typical (50–100 mM) levels for many crop species (Munns & James, 2003). Fields are often at their least saline at planting, during or after the rainy season. The most sensitive stage in faba bean was the pre-flowering vegetative phase, with less response during flowering

Table 2. Identified sources of resistance or tolerance to chilling or freezing stress

Species	Accession	Selection criteria	Reference
Chickpea	Derivatives of <i>C. reticulatum</i> and <i>C. echinospermum</i> ILC 8262 ILC 8617 FLIP 87-82C	Winter survival	Singh et al., 1995; Malhotra, 1998
Chickpea	SP1.563 Gully 940-26	Dry matter production in winter	O'Toole et al., 2001
Chickpea	ICCV88501	Yield following chilling of flowers	Sandhu and ArasaKesary, 2003
Chickpea	ICCV88516	Pollen tube growth at low temperature	Clarke and Siddique, 2004
Faba bean	Côte d'Or BPL 4628	Winter survival Controlled freezing tests	Duc and Petitjean, 1995
Faba bean	ILB 12 ILB 14 ILB 318 ILB 3187/Cixi Dabaican ILB 2999	Winter survival	Olszewski, 1996
Faba bean	Côte d'Or, Hiverna Göttingen winter bean population	Controlled freezing tests	Link and Arbaoui, unpublished
Lentil	LC 9978057 LC 9977006 LC 9977116 LC 9978013 ILL 759 ILL 1878 ILL 4400 ILL 7155 ILL 8146 ILL 8611 ILL 9832 Kafcas Cifci Uzbek	Winter survival rates Visual damage rating	Hamdi et al., 1996
Lentil	Balochistan Local ILL 5865	Controlled freezing test	Ali et al., 1999
Lentil	WA8649041 WA8649090 ILL 1878	Winter survival of parents and progeny lines	Kahraman et al., 2004a
Lentil	ILL 662 ILL 857 ILL 975 ILL 1878	Winter hardiness Early vigour Rapid ground cover	Sarker et al., 2002
Pea	EFB33 Unrra Württembergische	Survival in a harsh winter	Urbatzka et al., 2005

and pod-filling (Al-Tahir & Al-Abdulssalam, 1997). Short-term exposure to salinity may not be adequate to separate sensitive from tolerant lines, but long-term exposure leads to other problems due to shading of one line by another, differences in timing of transition to flowering, as well as physical space (Munns & James, 2003). Many workers stated that the best discrimination between sensitive and tolerant lines in hydroponic or sand culture was achieved at 'moderate' stress levels, which depended on the species concerned, being 25 mM NaCl for chickpea (Sadiki & Rabih, 2001), 75 mM for faba bean (Cordovilla et al., 1995) and either 70 or 75 mM for pea (Hernández et al., 2000; El-Hamdaoui et al., 2003; Gómez et al., 2004). Lentil responses varied with both growth stage and salinity level (Ameen, 1999), ranging from ILL 5582 (susceptible at flowering and sensitive based on seed yield) to ILL 5845 (tolerant at flowering and tolerant based on seed yield).

In a long series of experiments, Katerji et al. (2003) evaluated nine legume species, one per year, in a lysimeter at a range of EC values. Their best predictor of yield response was the 'water-stress-day index', which was the average of the difference in leaf-water potential between the saline and non-saline treatments through the growing season. This model was clearly not suitable for screening large numbers of genotypes, but it provided valuable data on typical response of the species concerned, including threshold EC values for the detection of damage and a slope of loss of yield as EC increased (Table 3). The critical value for salinity tolerance in lentil was earlier estimated as 4 dS/m (Saxena et al., 1994) but Katerji et al. (2003) evaluated it at only 1.7 dS/m (Table 3), using a different method and cultivar. Faba bean was found to be less sensitive to salinity than either chickpea or lentil, but these species were more sensitive than any others that these workers examined, including maize, barley and soybean, and

part of the difference was attributed to the prolonged flowering period in the cool-season legumes in contrast to the other crops (particularly cereals) examined (Katerji et al., 2003).

Numerous pot-based methods have been developed for assessing salinity response in different crops. River sand adequately reproduced field soil, water and temperature changes following an irrigation event whereas very fine silica sand did not (Wang, 2002). Munns and James (2003) described their preferred system comprising gravel-filled pots, supporting the plants and allowing aeration, set into trays which were cyclically sub-irrigated with saline solution introduced gradually after germination. This system has been used for cereals so far but could be applied to other crops. Serraj et al. (2004) screened 252 ICRISAT chickpea lines in pots filled with 7 kg of unspecified soil, irrigated with either tap water or 100 mM NaCl solution at sowing. Using the susceptibility index of Fischer and Maurer (1978), they identified 10 chickpea lines with potential salinity tolerance (Table 4). Similarly, Sadiki and Rabih (2001) screened 200 Moroccan chickpea lines in sand benches in a shadehouse, using 0, 25 and 50 mM NaCl. The best seven lines (determined by top dry weight, nodule mass, acetylene reduction assay and shoot nitrogen content) were tested in small plots again in larger on-farm plots. Three out-performed local controls, both in non-saline and especially in saline conditions, also showing greater nodulation (Table 4). Line MCA250 was the highest yielding line in the presence of salt and lost only 9% of its yield in comparison with the non-saline condition.

Physiological and biochemical tests

Salinity puts the plant under considerable oxidative stress. The levels of numerous enzymes which detoxify active oxygen species, including peroxidases and

Table 3. Salinity and boron-tolerance ratings of cool-season grain legumes

Species	Salinity tolerance (EC 8.2 dS/m) ^a	Boron tolerance (17 mg/L) ^a	Threshold salt sensitivity, EC _e (dS/m) ^b	Slope of response (% yield reduction per dS/m) ^b
Faba bean	Moderately susceptible	Susceptible	2.8	14.4
Chickpea	Susceptible	Susceptible	1.9	37
Lentil	Very susceptible	—	1.7	62
Pea	Very susceptible	Moderately susceptible		

^aFerreira et al., 1997.

^bKaterji et al., 2003.

Table 4. Identified sources of resistance or tolerance to salinity stress

Species	Accession	Selection criteria	Reference
Chickpea	SG-11	Seedling growth	Singh and Singh, 2001
	DHG-84-11	Sugar, starch, proline, phenol	Singh et al., 2001
Chickpea	ILC 1919		Soussi et al., 2003
Chickpea	ICC 10755	Salinity susceptibility index	Serraj et al., 2004a
	ICC 13124	Shoot dry matter production	
	ICC 13357		
	ICC 15406		
	ICC 15697		
	ICCV 92318		
	ICCV 92337		
	ICCV 95332		
	ICCV 95334		
	Jumbo 2		
Chickpea	MCA 103	Grain yield and nitrogen fixation	Sadiki and Rabih, 2001
	MCA 131		
	MCA 250		
Faba bean	Giza Blanca		Gaballah and Gomaa, 2004
	Giza 674		
Lentil	ILL6796	Biomass	Ashraf and Zafar, 1997
		Efficiency of K-utilization	
		Soluble sugars	
Lentil	NEL-2704	Germination	Mamo et al., 1996
		Plant growth	
		Yield	
Lentil	DLG-103	Germination	Rai and Singh, 1999
	LC-50	Plant growth, height, root length	
	LC-53	Nodulation	
	Sehore 74-3	Yield	

superoxide dismutases, were found to be elevated in the cytosol (Hernández et al., 2000), apoplast (Hernández et al., 2001) and chloroplasts (Gómez et al., 2004) of the tolerant pea cultivar in each experiment, but not in the intolerant line. Perhaps, this area offers potential for a rapid selection test, such as an antibody reaction, for use in plant breeding.

Leaf injury was scored rapidly, but had a high standard error, requiring up to 10 replicates (Munns & James, 2003). Shoot sodium content was quoted in many papers, along with $\text{Na}^+:\text{K}^+$ ratio and chloride content. In chickpea, an 8-fold increase in salinity (1.0–8.1 dS/m) resulted in a 1.5-fold increase in sodium and potassium content but a 13-fold increase in chloride (Rao et al., 2002). A realised heritability value of 0.37 in white clover (*Trifolium repens*) indicates that selection for low chloride content may be an appropriate strategy (Rogers et al., 1997).

Many authors reported proline accumulation in legumes under salt stress (chickpea: Ozcan et al., 2000; faba bean: Belkhodja, 1996; Trinchant et al., 1998; Heikal et al., 2000; Tammam, 2003; lentil: Soussi et al., 2003; Bandeoglu et al., 2004; pea: Fedina et al., 1993). Sugar concentrations also increased in response to salt stress (pea: Olmos & Hellin, 1996; faba bean: Ullah et al., 2001). In lentil and chickpea, total sugars increased only in the salt tolerant genotype (Ashraf & Zafar, 1997; Singh et al., 2001). Similarly, salt-tolerant faba bean cultivars had the highest leaf-soluble carbohydrate content under moderate salinity whereas sensitive genotypes had higher leaf-proline contents (Gaballah & Gomaa, 2004). Relative water content was also significantly reduced in salt-stressed faba beans, but no differences due to genotype were reported (Gadallah, 1999; Tammam, 2003). Katerji et al. (2003) found no correlation between osmotic adjustment, leaf

area and yield reduction in different legumes under salt stress.

Salt stress caused an increase in membrane damage in lentil leaf tissue (Bandeoglu et al., 2004). Under salt stress, ABA accumulated in pea leaves (Zdunek & Lips, 2001) and in soybeans, the tolerant genotype showed the higher ABA accumulation (Umezawa et al., 2001).

The nitrogen-fixing bacterium *Rhizobium leguminosarum* bv. *viciae* line 3841 failed to adsorb onto pea roots at 75 mM NaCl and also would not grow in culture at this salinity level (El-Hamdaoui et al., 2003). Additional boron and calcium were necessary to reduce these symptoms and to allow some nodulation and nodule function. Similarly, Rao et al. (2002) found that infection of chickpea root hairs by the rhizobium was greatly reduced in the presence of salinity. Cordovilla et al. (1995) took care to use a salinity-tolerant rhizobium strain in their work on salinity response in faba bean. This is a complex area for investigation, as the host genotype, the symbiont genotype and their interaction are all potentially responsive to the stress.

Waterlogging response

Waterlogging is often seen in autumn-sown crops, where autumn and winter rainfall greatly exceed evaporation and the plants are small, so transpiration rates are low. Furthermore, soils may have been compacted by sowing equipment. Some soil types are particularly prone to transient waterlogging (McDonald, 1995; Keneni et al., 2001). Where crops follow an irrigated crop such as paddy rice or cotton, there may also be a waterlogging problem, as in faba beans after rice in parts of China (Bond et al., 1994).

Soil waterlogging leads rapidly to hypoxia (low oxygen tension) and eventually to anoxia. Plant injury shows as wilting, cessation of growth, premature leaf senescence and nodule impairment (Marschner, 1995). Roots cease growth before shoots, so nutrient uptake ceases and nutrient concentrations are diluted in the

shoot (Barrett-Lennard, 2003). In the soil, nitrite, ferrous iron and other reduced ions can accumulate to toxic levels (Marschner, 1995).

Screening methods

Screening for waterlogging tolerance in the field generally involves comparison between raised plots (i.e., drained soil) and flat beds (McDonald, 1995; Keneni et al., 2001). In hydroponic or aquaponic systems in a controlled environment, further refinement is possible. Oxygen may be bubbled through the solution and compared with a nitrogen-bubbled system, to separate the effect of anoxia from other aspects of waterlogging (Aschi-Smiti et al., 2003) and ethylene may also be bubbled to promote the opportunity for development of aerenchyma (Marschner, 1995).

Formation of lysigenous cavities and aerenchyma is a waterlogging response found in many plants, including lentil (references in Erskine et al., 1994). Roots of pea cv. Alaska formed lysigenous cavities which functioned as aerenchyma, under conditions of high respiratory demand (Gladish & Niki, 2000). Intraspecific variation is known (Table 5) and other useful indications may be obtained from studies on other legumes. In the presence of high ethylene concentrations due to waterlogging, rhizobium infected aquatic and semi-aquatic tropical legumes by way of cracks in lateral root bases, instead of through root hairs as in terrestrial species (Goormachtig et al., 2004). Aerenchyma formation was correlated with the continuation of nodule activity in soybean (Shimamura et al., 2003) and in *Lotus* (James & Sprent, 1999). Soybean (*Glycine max*) cv. Aso aogari (Shimamura et al., 2003) and *Trifolium subterraneum* cv. Park (Aschi-Smiti et al., 2003) were considered to be exceptional in their respective species for their ability to produce aerenchyma when flooded, thus maintaining oxygen supply to the roots. Up to 15% of the root and stem-base volume in flooded soybean was aerenchyma (Bacanamwo & Purcell, 1999). Aerenchyma was effective over a distance of 10–20 cm

Table 5. Identified sources of resistance or tolerance to waterlogging

Species	Accession	Selection criteria	Reference
Chickpea	DZ10-4 × JG79-2-3-88	Duration of flood survival	Bejiga and Anbessa, 1995
Chickpea	Line 946-512	Survival when waterlogged at flowering	Cowie et al., 1995
Lentil	ILL 6439	Biomass, stomatal conductance	Ashraf and Chishti, 1993
	ILL 6778		
	ILL 6793		

Table 6. Identified sources of resistance or tolerance to extremes of boron concentration

Species	Accession	Selection criteria	Reference
Pea	NGB 1430 ^a NGB 2126	Boron accumulation in B-toxic soil; brown leaf spots	Bagheri et al., 1994
Lentil	ILL 2024 ILL 213A	Symptom score in B-toxic soil based on leaf spots and necroses	Hobson et al., 2003
Lentil	ILL 8009 ILL 5888 ILL 8010 ILL 2580	Yield in B-deficient soils	ICARDA, unpublished

^aNordic Gene Bank accession number.

and was therefore considered to be less useful in deep-rooted plants than in those with rapid adventitious root formation (Barrett-Lennard, 2003). Adventitious root formation is another important response to water-logging (Aschi-Smiti et al., 2003). In lentil, genotypes which were more resistant to flooding were characterized by a higher stomatal conductance compared to the more sensitive ones (Ashraf & Chishti, 1993), indicating a possible use as screening criterion.

Other mineral stresses

The limiting factor of some alkaline soils for crop growth is boron toxicity, which occurs primarily in arid areas and is reported in lentil growing regions of Australia, India, Pakistan, Iraq, Peru and Turkey. This is relatively easily screened by adding H_3BO_3 to the potting soil at 150 mg B/kg soil and scoring for the presence of brown necrotic spots on leaves (Bagheri et al., 1994). Significant variation in boron tolerance has been demonstrated in pea (Bagheri et al., 1994, 1996) and in lentil (Yau & Erskine, 2000; Hobson et al., 2003) (Table 6). Boron response in pea was attributable to two major genes, both acting in an additive manner to exclude boron from the roots (Bagheri et al., 1996). Although faba bean was listed as 'susceptible' to boron toxicity (17 mg/L, Ferreyra et al., 1997; Table 3), it performed well in South Australia, where high-boron soils are common.

Boron deficiency is a problem in other regions, such as the eastern Terai plain of Nepal, as well as northern Bangladesh, where the soil is frequently leached. Landraces from Nepal and Bangladesh were shown to be tolerant to B deficiency (Table 6), and accessions of West Asian origin were highly inefficient in B-deficient soils (Srivastava et al., 2000).

Conclusions

Broad abiotic stress resistance can be successfully combined with high yield potential, as shown by CIM-MYT's approach to wheat breeding (Braun et al., 1996) and is a target in the grain legumes (e.g., faba bean, Link et al., 1996). Many genes show greatly altered expression following different stresses (Figure 2) and it may be that the success of breeding for broad adaptation is due to the accumulation of the most 'useful' allele of some of these general stress-response genes. Very often, the highest yielding line in a stress environment is the highest yielding line in the non-stress control environment. It remains to introduce or enhance resistance to key abiotic stresses in a generally vigorous or adapted genetic background. This will be based on marker-assisted back-crossing. The natural limits to tolerance to any given abiotic stress have not been reached in the species under discussion here. Further levels of tolerance may be introduced from related species by conventional methods, as has been done for cold tolerance in chickpea, or may ultimately be derived

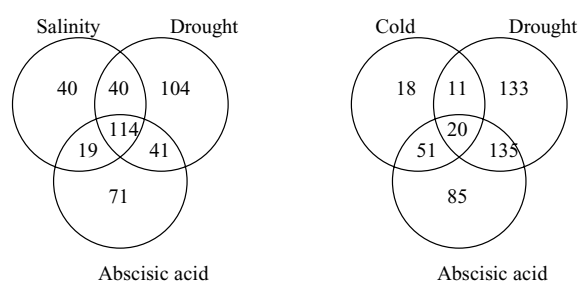


Figure 2. Numbers of genes showing at least 5-fold increases in expression in the presence of defined stresses in *Arabidopsis*. Adapted from Seki et al. (2003).

from unrelated species by genetic engineering methods, as discussed in other review papers in this issue. There is promising potential to identify candidate loci or alleles for stress tolerance, on the basis of recent findings in the model species *Arabidopsis thaliana*, *Medicago truncatula* or *Lotus japonicus*. Using this foreseeable, legume-derived, genomic progress for defining candidate genes may be more productive than the brute-force methods of high numbers of marker loci and association mapping. These modern methods will assist us in breeding highly stress-tolerant cool-season grain legume crops.

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