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*R*eview

Improvement of faba bean (*Vicia faba L.*) yield and quality through biotechnological approach: A review

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Faba bean (*Vicia faba* L.), an old-world grain legume, is grown approximately in 3 m/ha area world-wide from temperate, tropical to hot arid conditions. It is such a wonderful legume that it can excel even under adverse soil conditions; acidic or saline alkaline (pH 4.5 to 9.0). In favourable conditions, it gives very high yields, but low yield may result from biotic and abiotic stress. In India, it is still treated as minor legume. Genetic transformation based on *Agrobacteria* is possible. Several random amplified polymorphic DNA (RAPD) markers linked to a gene determining hypersensitive resistance to race 1 of the rust (*Uromyces viciae-fabae*) have been reported. Molecular breeding for resistance to broomrape, *Ascochyta* blight, rust, and chocolate spot have been obtained. The use of marker assisted selection (MAS) can complement conventional breeding by speeding up the selection of desirable traits and increasing selection efficiency. Recently, markers linked to a gene controlling growth habit or to select against traits affecting the nutritional value of seeds have also been reported. Lack of suitable cultivar can be easily overcome by application of modern tools and techniques. Several *in-vitro* techniques would be very useful for faba bean breeding. New techniques such as protoplast fusion, regeneration, and embryo-rescue assisted interspecific crossing could probably be introduced to *V. faba* L. to improve yield and quality. This review work examines the role of various techniques with reference to faba bean improvement.

Key words: *Vicia faba*, faba bean, grain quality, resistance breeding, nitrogen fixation, zero tannin.

INTRODUCTION

Faba bean is one of the oldest crops in the world. Globally, it is the third most important feed grain legume after soybean (*Glycine max*) and pea (*Pisum sativum* L). According to Food and Agriculture Organization (FAO), presently, it is grown in 58 countries. Despite the incredibility of the crop with its potential of serving the human society in India, it is categorized as minor, underutilized, less utilized, and not fully exploited (FAOSTAT, 2009). Nitrogen fixed by faba bean results in increased residual soil nitrogen for subsequent crops. It can also be used as green manure which has potential to fix free nitrogen at 150 to 300 kg N ha^{-1}. Faba bean is seen as an agronomically viable alternative to cereal grains. It is a good source of lysine rich protein as well as levodopa (L-3,4-dihydroxyphenylalanine (L-dopa)). Levodopa, a precursor of dopamine, can be potentially used as medicine for the treatment of Parkinson's disease. It is also a natriuretic agent, which might help in controlling hypertension. Persons consuming faba bean may suffer from favism (type of anaemia) if they lack an enzyme called glucose-6-phosphatage dehydrogenase (G6PD). Varieties containing zero tannin are now available, which can be directly

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Abbreviations: RAPD, Random amplified polymorphic DNA; **MAS,** marker assisted selection; **QTLs,** quantitative trait loci; **TFs,** transcription factors; **GUS,** ß-glucuronidase; **GFP,** green fluorescent protein; **EST,** expressed sequence tags; **DAP,** days after pollination; **CAPS,** cleaved amplified polymorphic sequences; **SCAR,** sequence characterised amplified region; **SNP,** single nucleotide polymorphism; **NBS,** nucleotides binding site; **LRR,** leucine rich repeat.

processed and fed to pig and poultry. Ability to adopt with par excellence as usual even under adverse growing condition at any stage of its life cycle coupled with its wide adaptability to a range of soil environment, productivity, high nutritive value, and usage in high value medicine, make it the strongest contender to be “king of crop”. It is very unfortunate in India and sub-continent, that despite the proven capability of this crop elsewhere, it is unable to grab its due respect and still categorized as unutilized/underutilized/minor/potential legume. It is very unfortunate that such wonderful crop is treated as a neglected and orphan crop in India. Only two varieties, namely “Pusa Sumit” and “Vikrant” (VH-82-1) have been released in India. This statement is more than enough to indicate its current status/role in Indian agriculture. The present situation also caution towards the urgent need to develop high yielding varieties for this crop. Perhaps, it is one of the most diversified crops which can perform better under changing climate situation.

STRATEGY FOR FABA BEAN IMPROVEMENT

The main objective of any faba bean crop breeding program is the development of improved breeding lines with an adequate resistance/tolerance to biotic as well as abiotic stresses. The use of model legumes for comparative functional genomics may bring some new perspectives and enhances faba bean breeding efforts (Rispail et al., 2010). In this way, identification of quantitative trait loci (QTLs) and candidate genes involved in stress tolerance and/or quality may be used to produce transgenic lines or can be applied to breeding programs e.g. marker assisted selection (MAS). Little is known about the functional correspondence of model legume genes and their putative faba bean orthologous.

Notwithstanding the lack of information, predictions can be made based on the sequence similarities between the relatively few *Medicago truncatula* and faba bean gene pairs existing between legume genomes, whereas, for highly conserved genes, favourable mutations observed in model legumes are likely to correspond to favourable alleles in faba bean, and for less conserved genes (that is, many transcription factors (TFs)), the relation is less reliable. Possible complications include: (1) Differences in gene copy number, (2) differences in transcript or protein abundance, and (3) differences in specific activity. Therefore, the information obtained in model legumes can be used as a guide to narrow down candidate genes, but proof can only come from functional studies, preferably in the homologous system. Once a series of candidate genes to improve a particular trait has been identified in one of the model legumes, a number of options are possible for exploiting this information in legume crops and particularly in faba bean breeding. The involved steps are: (1) confirmation of candidate gene function either directly in faba bean or indirectly in any of the model legumes, (2) identification of favourable alleles for selection, and (3) variety improvement by MAS or by transformation of an elite line. Several approaches have been developed to confirm candidate gene function at the biochemical and physiological level (Alghamdi, 2009). Originally, functional analysis of proteins was performed through two main techniques, protein over-expression and monitoring of promoter activity. Over-expression of a candidate gene is obtained by transferring the coding region of the gene under control of a strong promoter, such as the CaMV 35S into the plant, and function is assigned by scoring the phenotype of the resulting transformed line. Promoter activity analysis is performed by linking the promoter sequence to reporter gene such as the ß-glucuronidase (GUS) or the green fluorescent protein (GFP) to allow analysis of tissue-specific expression. Both procedures require gene transfer that is difficult in large seeded legumes. This limitation can often be short-cut by hairy root transformation that is easier to achieve, but only allows analysis of gene constructs in root tissue (Gresshoff and Delves, 1986). Albeit, with low efficiency, protocols for both *Agrobacterium tumefaciens* and *Agrobacterium rhizogenes* transformation have been established for faba bean and can be used for gene functional analysis in this species. Alternatively, the functional analysis could be performed in the model legumes *M. truncatula, Lotus japonicus* or soybean for which the transformation protocols are more efficient and rapid. In these model legumes, gene function can also be removed by modern molecular genetic techniques, including RNA interference (RNAi) and even targeting-induced local lesions in genomes (TILLING) (Kaimoyo and Van Etten, 2008). TILLING approach is also available for *G. max*, but not yet for faba bean; the difficulty being generation and maintenance of a large perfectly homozygous population for mutagenesis. Once the function of a candidate gene has been validated, identification of favourable alleles has to be performed. Defining patterns of synteny and collinearity between species by comparative genomic studies helps the identification of orthologous genes in genetically recalcitrant species as compared to model systems. Once a gene behind a given phenotype has been identified by a map-based cloning approach and validated, the orthologous gene in the other species can be isolated based on similar map position. There are several examples for this fruitful approach among the legume species where genes involved in symbiotic interactions have been identified (e.g. pea mutant’s sym19 - DMI2/NORK, sym2 - LYK3, and sym7 - NSP2 (Kalo et al., 2005). The syntenic map position of the dwarf phenotype in diploid alfalfa (Msdwf1) and pea (le) and the genomic resources in *M. truncatula*, enabled the identification of a gene encoding a gibberellin 3-β-hydroxylase (GA3ox) required for normal growth habit in diploid alfalfa.

These examples clearly demonstrate the two-way utility and application of molecular markers and the identified

orthologous regions between the genomes of reference and crop legumes. Tools developed in model species can facilitate the identification of agronomically important genes (QTLs, genes involved in nutrient quality and quantity, biotic and abiotic stresses, etc.) and marker-assisted breeding programs in target organisms while the accumulated biological knowledge in crop species can contribute the understanding of biological processes. Alternatively, selection of favourable alleles of the gene of interest can be found using the EcoTILLING approach that allows the detection of allelic variants of a candidate gene in natural populations for their subsequent phenotyping for the trait in question. Finally, the favourable allele can be transferred to elite faba bean cultivars by MAS or genetic transformation (Horst et al., 2007).

Seed biology of *M. truncatula* is essentially very similar to that of the major temperate crop legumes, pea, and faba bean, but differs in that the major carbon reserves are lipids, rather than starch, which is present only in trace amounts in the mature seed. The *M. truncatula* seed also contains about 10% of endosperm material at maturity, unlike pea or faba bean in which this layer is reabsorbed during development. As *M. truncatula* was not bred for grain consumption, its seeds are also relatively small with a relatively high proportion of cell wall material and a low harvest index. Proteins represent the major class of storage compounds in *M. truncatula* seeds, followed by lipids, with only trace quantities of starch, whereas proteins and oils are co-ordinately synthesized during seed filling, the non-starch carbohydrate fraction (mainly *trachyose*) accumulates only at the end of seed maturation, when seeds are acquiring desiccation tolerance. Fatty acid and sugar compositions are similar to those of pea and other grain legumes. Thus, with certain caveats, the *M. truncatula* seed is a good model for identifying genes important in regulating seed composition in grain legumes.

IDENTIFICATION OF GRAIN QUALITY CHARACTERS

The availability of a comprehensive expressed sequence tags (EST) database has allowed a large-scale identification of genes putatively encoding *M. truncatula* seed proteins that have been subsequently confirmed by seed protein separation and Matrix-Assisted Laser Desorption/Ionization-Time-of-Flight (MALDI-TOF) analysis, some of which are candidate genes for quality traits. The major *M. truncatula* storage proteins are the 7S (vicilin and convicilin-type) and 11S (legumin-type) globulins, with similar amino acid compositions to those of other grain legumes, notably being poor in sulphur-containing amino acids. The storage proteins accumulate sequentially during seed filling, the vicilin *s* at 14 days after pollination (DAP), followed by the legumins (16 DAP) with the convicilins accumulating last (18 DAP). Among the proteins identified at different developmental stages, several enzymes and other proteins play key roles in the seed. For example, cell division-associated proteins were expressed during the differentiation phase preceding seed filling. Storage protein accumulation was accompanied by the expression of putative chaperonins and protein disulphide isomerases. During this phase, two PV100-like polypeptides also accumulate giving rise to a trypsin inhibitor and a cytotoxin-related peptide upon processing, which are important targets for breeding as their elimination could improve nutritional quality of legume seeds. Starch accumulates only transiently in *M. truncatula* seeds, in contrast to the starch-rich pulse, pea, and faba bean, but similarly to the situation in soybean, starch remobilisation is the contributing carbon source for oil biosynthesis. Certain starch-remobilisation enzymes (starch synthase, sucrose synthase, and triose phosphate isomerase) were transiently expressed 16 to 24 DAP, concomitantly with proteins involved in photosynthesis, supporting the hypothesis that photosynthesis in the embryo provides energy for lipid biosynthesis, which may also recycle fixed CO_2 .

Seed development involves the interplay of several tissues; the developing embryo is surrounded by the endosperm, and the two organs are embedded in the maternal integument. The role played by the embryo-surrounding tissues in legume seed reserve accumulation has been investigated genetically for pea and soybean, indicating significant maternal effect in seed filling. To study these interactions in more detail and get access to the genes involved, gene expression in these tissues has been analysed at the proteome and transcriptome levels. A general observation is that the pattern of proteins and transcripts expressed in the embryo, endosperm, and integument is very specific for each cell type, with little overlap. One of the major findings was an extensive compartmentalization of amino acid metabolism between seed tissue components that may favour storage product accumulation. Of particular interest is the compartmentalization of enzymes of sulphur amino acid biosynthesis, observed for both methionine and cysteine, as these are limited in grain legumes. The dependence of the embryo's nutrition on the maternal tissue was also demonstrated directly by an *in vitro* culture experiment in which embryo development on nitrogen nutrient-free medium with and without the surrounding tissue was compared. Embryos grown without nitrogen source aborted, whereas embryos grown in the presence of the surrounding endosperm and integument developed normally and accumulated reserve proteins, presumably due to nitrogen remobilisation from maternal tissues. This remobilisation of a temporary nitrogen store requires proteolysis, and candidate proteases with appropriate expression kinetics have been identified in endosperm and seed coat tissues. Seed developmental programme is under tight transcriptional control, and there is evidence from other plant systems that an important class of loci regulating seed composition corresponds to TFs.

To identify TFs expressed in developing *M. truncatula*

seeds, expression of more than 700 TF sequences was monitored by quantitative real-time polymerase chain reaction (PCR) throughout seed development. By clustering the data of TF expression with storage protein expression profiles previously obtained, candidate factors potentially controlling the major storage protein groups were identified. In parallel, a biochemical approach analysing the nuclear proteome led to the identification of several putative regulatory proteins, the functions of which remain to be determined by reverse genetics. Identified genes and proteins from all these studies may serve as quality markers potentially transferable to crop legumes for breeding once their involvement in seed quality is determined and polymorphism for these traits are found. A survey of natural variations in seed protein complements carried out on 50 diverse *M. truncatula* ecotypes or cultivars indicated a high degree of polymorphism in protein composition and large variation in protein content (33 to 46%). Clustering of genotypes according to similarity in one-dimensional protein profiles allowed structuring into classes that corresponded to 4 species groups within the *M. truncatula* species complex. This classification has allowed the selection of recombinants inbred lines (RIL) parents for maximizing variation in protein content and type in the populations to be examined, and mapping of QTLs is in progress. A survey of QTLs for traits affecting vegetative plant development and seed yield and content in pea revealed the importance of genes determining plant architecture in controlling seed yield and protein content. It would appear likely that the homologous loci in faba bean have the same properties, and therefore, these should form part of selection schemes. Apart from selection for seed size and seed number per pod, quality breeding in faba bean is concentrated on the reduction/elimination of the anti-nutritional factors condensed tannins, *vicine* and *convicine*, responsible for favism, a severe digestive disorder in susceptible individuals, which reduce nutritional value of faba bean. Cleaved amplified polymorphic sequences (CAPS) markers have been obtained for the convicine locus vicine-convicine (v-c), and sequence characterised amplified region (SCAR) markers for the *tannin* loci zt-1 and zt-2 for use in introgression of favourable alleles in breeding selection (Gutierrez et al., 2006). Linked molecular markers, such as the aforementioned markers, may be subject to recombination with the trait of interest. With the sequence data available from *M. truncatula*, there should be a possibility of identifying genes encoding the responsible enzymes, and thus, obtaining non-recombining, and hence, more reliable single nucleotide polymorphism (SNP) markers within the gene itself. The development of inbred lines, perhaps based on the closed flower mutation, would facilitate genetic analyses.

BREEDING FOR RESISTANCE TO BIOTIC STRESSES

Grain legume and in particular faba bean are challenged by many pathogens and pest, including bacterial, virus, and fungal diseases as well as infection by nematodes and some parasitic plants which strongly affect crop yield worldwide. Genetic resistance is considered the most desirable method since it is the most cost effective and environment-friendly than the use of chemicals. Thus, many resistance sources and their associated QTLs have been found in different grain legumes, including faba bean. However, the long genetic distance existing in most cases between the identified genetic markers and the resistance QTLs, the common lack of codominant markers and general lack of knowledge about resistance mechanisms in legumes limit greatly the use of genetic markers to confer resistance to grain legumes. The model legumes *M. truncatula* and *L. japonicus* are affected by many of the pathogens and pest limiting faba bean yield. Thus, they offer a great opportunity to improve the knowledge of resistance mechanisms against faba bean pathogens and identify effective resistance genes against them. Fungal and oomycete pathogens are the most diverse group of pathogens and they cause the most dramatic damages on legume yield worldwide (Gonzalez et al., 1995).

Annual *Medicago* and *M. truncatula* in particular are strongly affected by a wide range of foliar and soil-borne necrotrophic fungi which makes of *M. truncatula* a promising model to study the plant–necrotrophic fungi interaction. Several studies revealed that *M. truncatula* is a potential host not only of necrotrophic fungi, but also of several biotrophic fungal and oomycete pathogens, including *Aphanomyces euteiches*, *Curvularia trifolii*, *Erysiphe pisi*, *Fusarium* species, *Leptosphaerulina trifolii*, *Mycosphaerella pinodes*, *Phoma medicaginis*, *Peronospora trifoliorum*, and *Uromyces striatus*. In most cases, screening of germplasm collections of *M. truncatula* allowed the identification of a wide range of differential responses to the pathogen from highly susceptible to resistant. This serves as bases for the characterisation of underlying resistance mechanisms at the cellular and molecular levels as well as for the identification of defence genes and QTLs responsible for resistance. *Truncatula* resistance against *P. medicaginis* and *C. trifolii* was found to be controlled by single major genes, named rnpm1 and RCT1, respectively. These major genes localized at the top of the linkage group 4 in a region containing a cluster of several nucleotides binding site (NBS)-leucine rich repeat (LRR) proteins that are often plant resistance (R) genes. Interestingly, the RCT1 gene of *M. truncatula* has been successfully transferred to alfalfa to confer anthracnose resistance.

Resistance to *A. euteiches, M. pinodes, U. striatus, P. trifoliorum* or *E. pisi* of pea is controlled by different defence mechanisms. For instance, screening of an USDA collection of *M. truncatula* germplasms for *E. pisi* resistance indicated that resistance to powdery mildew was controlled by papilla formation, by early hypersensitive response and also by post-haustorial mechanisms.

Mapping of the QTLs controlling resistance to these fungal pathogens in *M. truncatula* is now underway.

In parallel, the transcriptomic and proteomic approaches developed for this model legume are being used to understand the molecular components and to identify candidate genes involved in *M. truncatula* defence against these fungal pathogens. For instance, a substractive suppression hybridisation (SSH) library indicated that pathogen-related (PR) 10 proteins and proteins associated with abscisic acid signalling play important roles in the *M. truncatula* resistance against *A. euteiches*. The crucial role of PR10 in *A. euteiches* resistance was confirmed by comparative proteomic and gene silencing approaches, which indicated that PR10 silencing led to increased resistance by antagonist induction of other PR genes. Comparison of the proteomic profile of several *M. truncatula* lines with varying levels of resistance also identified other proteins potentially involved in *A. euteiches* resistance, such as proteasome alpha subunits.

Comparison of expression profiles of 92 defence-related genes by macroarray between a resistant and susceptible line of *M. truncatula* at key steps of *C. trifolii* infection also highlighted the important role of PR proteins and in particular PR10 in resistance. As expected, this analysis indicated that a large proportion of genes present on the macroarray membrane were upregulated in the resistant *M. truncatula* line, while these genes were mainly down regulated in the susceptible line. Microarray analysis of several *M. truncatula* genotypes with different defence mechanisms against *E. pisi* allowed the identification of a set of genes involved in defence mechanisms (Kuster et al., 2004). Post-genomic approaches are also being applied to tackle other fungal diseases, such as *M. pinodes* and *U. striatus*. Legumes are also affected by bacterial pathogens. In particular, *M. truncatula* can be infected by the causing agent of the bacterial wilt disease, *Ralstonia solanacearum*, which also infects a large number of crops including tomato, potato, and cultivated legumes, such as faba bean. A recent study showed that one *M. truncatula* line, F830055, susceptible to *C. trifolii* and *P. medicaginis*, was resistant to most *R. solanacearum* isolates. A major QTL was mapped on chromosome 5 and two minor ones on chromosomes 3 and 7 that may be helpful for MAS (Julier et al., 2007).

Nematodes are also an important cause of yield losses in legumes. Interestingly, *M. truncatula* and *L. japonicus* have been shown to be susceptible to most nematodes affecting legumes. For instance, *M. truncatula* can be colonised by the stem nematode *Ditylenchus dipsaci,* causing disease in many legumes, such as alfalfa, pea, and faba bean. By screening *M. truncatula* germplasm collection, several resistant and susceptible *M. truncatula* lines were identified that will surely allow a better understanding of stem nematode-legume interaction. *L. japonicas* and *M. truncatula* are also infected by different root-knot and cyst nematodes belonging to the *Meloidogyne* and *Heterodera* genera. In *L. japonicus*, root-knot nematode and rhizobium interactions may share common pathways. Indeed, it was found that *L. japonicas* mutants deficient for nitrogen-fixing symbiosis establishment were more resistant to *Meloidogyne incognita* than the wild type, while a hypernodulating mutant was infected to a higher extent by the nematode. On the other hand, screening of *L. japonicus* ecotypes revealed differential infection responses according to the ecotype ranging from susceptible to resistant to this nematode. Such genetic diversity is being used to map and identify genes and/or QTLs involved in root-knot nematode resistance. Although, less studied, legumes are also under the thread of viruses. Despite the damage they cause, very little is known about virus resistance mechanisms and nearly no studies have aimed at the characterisation of virus resistance in the two model legumes, *M. truncatula* and *L. japonicus*. The only report published to date indicated that *L. japonicus* could be infected by Arabis mosaic virus and tobacco ringspot virus, while it was resistant to most legume infecting viruses. Due to their economic importance, virus diseases have been studied more in soybean, thanks to its relatively small genome and the development of genomic tools begins to be considered as the third model legume. In this species, several resistance genes to the soybean mosaic virus have been identified and pyramidized in a single cultivar.

In semi-arid regions worldwide, including Southern and Eastern Europe, North and East Africa, and the Middle East, parasitic plants of the *Orobanche* and *Phelipanche* species, including *Orobanche crenata, Orobanche foetida*, *and Phelipanche aegyptiaca (syn. O. aegyptiaca)* drastically decrease legume yield. *M. truncatula* has been recently proposed as a model to study the interaction of *Orobanche* spp*. legumes*. *L. japonicus* can be infected by *P. aegyptiaca*, but shows incompatible interaction against *Orobanche minor, Striga hermonthica* or *Striga gesnerioides*. Even when *L. japonicus* is not infected by *O. minor*, its root exudates have strong stimulatory activity on *O. minor*, as well as on *O. crenata, Orobanche densiflora, P. aegyptiaca*, and *P. ramosa (syn. O. ramosa*) seeds (Kahal et al., 2003). To improve our understanding of the *M. truncatula–O. crenata* interaction, a SSH library has been created, allowing the identification of around 300 candidate genes for *O. crenata* defence. In addition, a microarray analysis of the *M. truncatula* genes regulated in response to *O. crenata* was recently performed on the M16kOLI1 microarray platform. A comparison of two-dimension proteomic profile of two *M. truncatula* genotypes varying in their level of resistance against *O. crenata* was also performed. Preliminary analysis of the comparison of the transcriptome of two *M. truncatula* genotypes with different resistance mechanisms indicated significant changes in the steady-state level of many transcripts belonging to several functional categories, including pathogen-induced genes, such as pathogenesis-related (PR) genes, hormone - associated

genes, and TFs. These analyses also revealed the activation of both the salicylic acid and jasmonate defense-pathways. These preliminary results support the previously established results and should prove useful to identify potential candidate genes for crop improvement. These candidate genes should be validated through functional analysis. Validated candidates may then be used for genetic improvement of crop either directly through genetic transformation or indirectly by MAS.

BREEDING FOR RESISTANCE TO ABIOTIC STRESSES

Global climate change predictions suggest new scenarios with larger arid areas and extreme climatologic events. Thus, it is essential to understand how plants respond to different abiotic stresses in order to improve crop performance. This difficult task can only be achieved by integrating conventional breeding and biotechnological approaches. However, most legume crops are not easily amenable for molecular and genetic studies. To circumvent this limitation knowledge gained on the two model legumes, *M. truncatula* and *L. japonicus* may be further used to understand the responses to abiotic stresses in other legumes, such as faba bean (Handberg et al., 1992). Among the numerous environmental constrains affecting crop yield, drought is considered the most limiting factor with important economic consequences. Faba bean is rather sensitive to drought. It is said to be specially susceptible after flowering, whereas a mild water shortage during flowering may be preferable to plenty of water in order to limit vegetative growth and stimulate early reproductive growth (Grashoff, 1990). *M. truncatula* is quite a drought-tolerant plant species compared to grain legumes, such as pea or soybean. Based on physiological and biochemical studies, *M. truncatula* responses to drought appear to be similar to those described in this study. The relative drought tolerance of *M. truncatula* has been shown in a recent study, where moderate water deficit had only a slight significant effect on plant biomass, presenting some differences among cultivars/ecotypes. Tolerances, showing that under mild drought conditions, *M. truncatula* plants were able to avoid leaf dehydration and under severe drought stress, plants maintained significantly high net CO_2 fixation rates. Particular emphasis has been laid on the regulation of symbiotic nitrogen fixation (SNF) under drought stress in nodulated legumes.

Analysis in *M. truncatula* suggests that the drought-induced down regulation of sucrose synthase is not mainly responsible for the inhibition of SNF; similarly, to observations in *Medicago sativa*. Additionally, the response to drought at the nodule level has been recently analysed under a proteomic perspective, where new marker enzymes, such as plant methionine synthase and bacteroid serine hydroxymethyl transferase were identified (Larrainzar et al., 2007). Regarding *L. japonicus*, there is an accumulation of proline and oxidative damage in leaves upon different water deprivation treatments; although, the first studies analysing the response of this legume to water deficit have started to emerge, most of the published work so far is based on other *Lotus* species. Plant responses to salt stress have been extensively analysed with an especial emphasis on the role played by different osmolytes in homeostasis maintenance. Some compounds, such as proline betaine, trehalose, trigonelline, and *a* pyridine betaine, have been reported to play a role in the response to salt stress of different legumes. Furthermore, *proline* accumulation has been shown to enhance SNF during salt stress in *M. truncatula* (Gruber et al., 2009). In a recent functional analysis, a general increase in the steady-state level of many amino acids, sugars, and polyols, with a concurrent decrease in most organic acids in response to gradual salt stress in *L. japonicus* leaves. On the other hand, molecular approaches have been applied to examine the response of *M. truncatula* and *M. sativa* under salinity leading to the identification of several TFs related to the plant root response to salt stress. The effect of low temperatures on plants has also received considerable attention. Unfortunately, little is known about the response of legumes to this type of stress, as most of the published reports are based on model plants such as *Arabidopsis thaliana*. Plant cold acclimation is a complex process, which involves the specific expression of cold-induced genes to stabilize membranes against freeze-induced injury. This group includes genes encoding late embryogenesis-abundant proteins, enzymes required for osmolyte biosynthesis, antifreeze proteins, chaperones, and detoxification enzymes, under the control of several cold-induced TFs (Hekneby et al., 2006). Based on the information available, it appears that *M. truncatula* exhibit a poor freezing tolerance, when compared with other annual legumes. This might be due to an ineffective cold acclimation process and low starch reserves in this species. Interestingly, the *M. truncatula* ZFP1 gene, encoding a root-enhanced zinc finger protein with high similarity to a soybean cold-inducible protein, is not regulated by low temperature, suggesting a different physiological function of this protein in both legume species. Some promising results for low temperature legume breeding have been obtained by transgenic expression of an iron-superoxide dismutase in alfalfa, resulting in an enhanced winter survival. Superoxide dismutase enhances tolerance of freezing stress in transgenic alfalfa (*M. sativa* L).

Flooding is another environmental stress that negatively influences germination, seedling establishment, and plant development, as it causes a limitation in the flux of oxygen to support plant respiration. Besides the activation of alcohol and lactic fermentative pathways, flooding stress on *M. truncatula* seedlings induces activity of mitochondrial alanine aminotransferase and glutamate dehydrogenase which may contribute to the maintenance

of the redox balance during fermentative growth. The involvement of non-symbiotic haemoglobins in flooding stress adaptation has been shown in *L. japonicas (*Jiang and Gresshoff*,* 1997), whereas promoter analysis carried out in faba bean suggested that leghemoglobins were not induced upon hypoxia. In the context of Grain Legumes Integrated Project (GLIP) European project, abiotic stress tolerance has been focused on species, such as *M. truncatula*, pea, and chickpea leading to identification of factors potentially involved in abiotic stress adaptation and tolerance. The involvement of some genes in abiotic stress response has been already analysed in different legumes. For instance, alfalfa over-expressing chloroplasttic MnSOD showed lower cold-induced membrane injuries; although, these transgenic lines did not present better tolerance to drought stress. The transcriptional regulator, Alfin1, over-expressed in alfalfa was shown to regulate endogenous NaCl-inducible gene expression, resulting in salinity tolerance. Similarly, a drought-responsive AP2-type TF induced several wax-related genes resulting in increased drought tolerance when over-expressed in alfalfa.

BREEDING FOR NITROGEN FIXATION

Nodulation is initiated by plant roots exuding flavonoid molecules into the soil. This attracts rhizobia to the roots and concomitantly stimulates them to synthesize a lipochito-oligosaccharide signalling molecule called Nod factor (NF). Using the model species *L. japonicus* and *M. truncatula*, and a predominantly mutant-based approach, many of the genes required for nodule development have now been elucidated. This includes genes encoding transmembrane LysM-type receptor kinases believed to be required for NF perception: LjNFR1 and LjNFR5 in *L. japonicus*, and MtNFP, MtLYK3, and LYK4 in *M. truncatula*. Subsequent to perception, NF signalling continues through a NBS-LRR receptor kinase, called LjSYMRK / MtDMI2. The signalling cascade then progresses via a number of genes, including those encoding potential potassium ion channels, MtDMI1, LjCASTOR, and LjPOLLUX, putative nucleoporins, LjNUP133 and LjNUP85, a calcium-calmodulin-dependent proteinkinase, MtCCaMK, a cytokinin receptor, LjLHK1/MtCRE1, and finally, TFs, including MtNSP1, MtNSP2, MtERF, and LjNIN. These genes are all required for nodulation; the loss of any results in reduced, or a complete lack of nodule formation (Hoffmann et al., 2007).

ZERO TANNIN FABA BEAN VARIETIES

High tannin (10% or higher seed tannin levels) cannot be fed at high levels to these livestock. In the 1970's, normal high tannin (10% or higher seed tannin levels) was grown as a silage protein crop (for cattle) and still some were even marketed to Egypt and other countries for human consumption Grade 3 or better. Zero tannin (less than 1% seed tannin level) is now available, which can be directly processed and fed to swine and meat poultry. These older tannin types originated from Europe (Germany and Netherlands) and tannin types are still grown as a livestock feed in Europe, because most of the feeds are pelletized and the heat in pelleting destroys the tannin problem. The feed quality of zero tannin faba beans is excellent and the production factors for this crop are quite simple and easy to obtain. The large potential of the new zero tannin faba beans (variety "Snowbird") are mainly as a feed for swine and meat poultry. "Snowbird" faba bean is a medium maturing cultivar (110 to 120 days to maturity) based on early seeding. Seed size is approximately 550 g/1000 seeds (about 2 times larger than normal field pea seed size).

To produce tannin free faba bean, the field should be free from perennial weed. Use only recommended dose of fertilizer especially in case of nitrogen. Sowing should be done at optimum soil moisture condition; if needed, pre sowing irrigation should be done. Square planting should be preferred keeping 30 cm apart with 3 to 4 cm depth (Martin et al., 1991). Sowing should be done in a manner that 4 plants per square foot must be maintained. Proper inoculants and a small amount of phosphate must be added to the total fertilizer applied in most fields. Crop weed should be made free and if needed proper herbicide, namely, Reglan, etc., should be applied in appropriate quantity and manner. Harvesting should be done by straight cut or direct reaping, when seed moisture is about 18 to 20%. Harvested crop should be left in the field to dry up to the level of 16% moisture.

CONCLUSIONS AND FUTURE PROSPECTS

The recent advances in our understanding of the biochemical and genetics in the integration of highly efficient breeding techniques with the power of biotechnological tools contribute to improvement of minor legume crops, such as faba bean. Lack of suitable cultivar can be easily overcome by the application of modern tools and techniques. Several *in-vitro* techniques would be very useful for faba bean. Based on the forgone discussion, it is clear now that for further improvement in faba bean, emphasis should be given on the following aspects:

1. Characterization of phenological, morphological, physiological, and biochemical traits of faba bean that will contribute its adaptation in target environments.
2. Characterization of the effect of gene and QTLs for selecting suitable genotypes with resistance to biotic and abiotic stresses.
3. Defining precisely the targets abiotic stress prone environments and delineate the predominate type of stress and the faba bean varieties preferred by farmers.
4. Using simulation modelling and system analysis to evaluate crop response to major abiotic and biotic stress pattern.

5. Harnessing functional genomics and reverse genetic tools to understand the genetic control of the relevant traits.
6. Developing flexible and site specific crop management options with a focus on faba bean crop establishment, weed management, and nutrient management.
7. Testing the advance molecular breeding products in well managed screening facility and in farmers' participatory multi-location trials.

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