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### Strategies for faba bean improvement growing under severe conditions and in the presence of a wide range of disease

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### Abstract

Successful production of faba bean crops under severe conditions and in the presence of a wide range of disease-causing fungi, parasitic weeds, nematodes, insects, mites and other pests depends on the integration of genetic resistance, hygienic management, monitoring of the target organisms and timely application of appropriate chemical and biological treatments. This paper reviews the strategies developed to enhance growth of faba bean, the limits and possible solutions. Control methods are being developed that comprise agronomical management techniques, chemical and biological control methods, genetic and induced resistance. However, the main concern is that to date, no single method of control provides complete protection against these pathogens and parasites. For that reason, an integrated approach is needed in which a variety of such techniques are combined, in order to maintain faba bean production under severe conditions. For inducing genetic diversity the use of ionizing radiation especially gamma rays, is well established. Induced mutations have been used to improve major crops which are seed propagated. Since the establishment of the Joint FAO/IAEA Division of the Nuclear Techniques in Agriculture, more than 1800 cultivars obtained either as direct mutants or derived from their crosses have been released worldwide in 50 countries. In the presence of regression of faba bean culture in the world caused by selection pressure on the pathogens and pests, creating new varieties, continued breeding for novel resistance genes, development of new selective chemicals, screening for new biocontrol agents and the design of new management strategies will all be necessary.

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## **K**EYWORDS

Faba bean: Pests and diseases: Induced mutations; Integrated crop protection; Genetic diversity; Gamma ray.

# Review INTRODUCTION

Faba bean is widely used in the Mediterranean region as source of protein in both human and animal nutrition<sup>[64]</sup>. The nutritional value of field bean has been traditionally attributed to it high protein content<sup>[24]</sup>. It is also a good source of sugars, minerals and vitamins. Thus, the chemical analysis of this legume reveals a 50-60% content of carbohydrate, which is mainly constituted by starch, while the proportion of lipids is relatively low at about 1-2.5% with oleic and linoleic acids representing about 75% of fats. Faba bean also contributes to farmer's income and improves the soil fertility through biological nitrogen fixation.

Despite all these beneficial aspects, the area and the production of legumes in Tunisia have not increased in the last years. Diseases and pests have been reported as recurrent problems in Tunisia<sup>[59]</sup>. This was highlighted during many seasons, where the majority of faba bean crop was devastated by chocolate spot incited by Botrytis fubae. Nematode (Ditylenchus dipsuci), rust (Urorrzyces fubue) virus diseases and root rot (Rhizoctoniu spp.) were also present<sup>[50,57]</sup>. Chocolate spot was identified in almost all the areas covered by the survey including the semi-arid and arid areas of the central and southern parts of the country where the climatic conditions are normally not conducive for disease development. Aphids and others insects such as Sitona spp. and stem borer (Lixus algirus) cause some damage to faba bean<sup>[6]</sup>. The presence of Orobanche spp. in some faba bean growing areas is considered as a limiting factor to the expansion of the crop<sup>[58]</sup>.

Genetic resistance is considered the most desirable control method since it is more cost effective and environment friendly than the use of chemicals. Gamma irradiation was found to increase plant productivity. In this connection, Jaywardena and Peiris<sup>[52]</sup> stated that gamma rays represent one of the important physical agents used to improve the characters and productivity of many plants (e.g. rice, maize, bean, cowpea and potato). Gamma rays belong to ionizing radiation and interact to atoms or molecules to produce free radicals in cells. These radicals can damage or modify important components of plant cells and have been reported to affect differentially the morphology, anatomy, biochemistry, and physiology of plants depending on the

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irradiation level. These effects include changes in the plant cellular structure and metabolism, e.g., dilation of thylakoid membranes, alteration in photosynthesis, modulation of the antioxidative system, and accumulation of phenolic compounds<sup>[62,63,111]</sup>. Radiation mediated in vitro mutagenesis and selection has been successfully used to improve agronomic traits such as salinity and drought tolerance in different crop plants<sup>[11,33,89,115]</sup>, advocating that tissue culture selection is useful to select stress-tolerant clones. Several works have shown that mutagenesis with gamma rays can be successfully used to develop new lines useful for breeding, such Sweetpotato, Grass pea<sup>[84]</sup>, Cocoyam Xanthosoma sagittifolium<sup>[12]</sup>. A major aim for any crop breeding program is the development of good quality lines with an adequate resistance/tolerance to yield-reducing stresses.

The combination of genetic resistance, hygiene and monitoring of crops for threshold levels of infestation, allows the most economic and effective use of chemical controls with the result that economic yields can be maximized. In this paper we review strategies for faba bean improvement growth under severe conditions and in the presence of a wide range of disease-causing fungi, parasitic weeds, nematodes, insects, mites and other pests.

### **BIOTIC STRESS**

### Water and salinity stress

Growth of faba bean is very sensitive to water stress<sup>[58]</sup>. That sensitivity is a result of its maximum depth of rooting is relatively shallow, approximately 0.9 m, and its disability to adjust osmotically to water stress<sup>[98]</sup>. Furthermore, water stress has a determinant effect on faba bean vegetative growth, as well as reproductive growth<sup>[77]</sup>. The early podding stage of development was the most sensitive to water stress, caused a reduction in faba bean yield by 50%<sup>[82]</sup>.

Salinity is a worldwide problem in irrigated areas<sup>[55]</sup>. In the Mediterranean area, the percentage of irrigated soils affected by salinity amounts to about 20%, varying from country to country between 7 and 40%<sup>[37]</sup>. Al-Tahir and Al-Ab dulsalam<sup>[45]</sup> results indicated that faba bean was more sensitive to salinity during the veg-

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etative stage and less sensitive at later stages. In faba bean water salinity significantly reduced the grain yield and grain number but did not affect grain weight. Salinity significantly reduced the dry weights of both faba bean's shoots and roots, shoots appeared to be more sensitive to salinity than roots<sup>[26]</sup>.

### Nitrogen deficiency

Faba bean deliver an important ecosystem service to cropping systems via its ability to symbiotically fix atmospheric  $N_2^{[53]}$ Dyke and Prew<sup>[31]</sup> reported that faba bean roots and stubble contributed 44–50 kg N/ha to the requirements of the following crop in a temperate climate. Provided the soil contains sufficient populations of effective rhizobia faba bean can accumulate nitrogen both from soil and the atmosphere. The relative contribution from each source to satisfying faba bean's nitrogen requirements for growth will be heavily influenced by the concentrations of available soil mineral N in the rooting zone<sup>[53]</sup>.

### Agronomic management methods

In regions where terminal drought is a regular occurrence, the length of the critical growing period may be minimized by selection of varieties with an appropriate phenology or by adoption of appropriate faba bean management strategies. These strategies of drought escape are not as successful when transient drought occurs, with unpredictable timing, earlier in the growing season. Early sowing and vigorous growth reduces soil evaporation so, by sowing earlier, the time to flowering and pod fill may lessen, thereby reducing the effect of terminal drought<sup>[58]</sup>.

### Chemical and biological methods

Concerning drought and salt tolerance, this character reported in several genotypes of faba bean may be important in some Mediterranean zones. It has been considered by a few physiologists in relation to the tolerance of the *plant-Rhizobium* symbiosis<sup>[21,35]</sup> but not specifically by breeders.

Biotic and abiotic stresses are not controlled effectively by traditional control strategies, and usually the used control method is environmentally hazardous. The control of these stresses has been the aim of many research programs, but success has been limited. A few varieties of crops have an inherent tolerance. The best long-term strategy for controlling stresses is through the breeding of resistant genotypes. Little work has been done on resistant varieties. For the herbicides they are of little use with parasitic mistletoes, and few host species show significant resistance useful in a breeding program. Despite many years of hard work by plant breeders, resistant cultivars of most crops are not available: breeding for parasitic plant resistance is very difficult. Development of effective genetic engineering strategies for resistance to parasitic weeds require identification of 1 genes whose products are selectively toxic and inhibit parasite growth and 2 promoter sequences that optimize expression of such toxins.

### FUNGI

Many fungi have been described, although only a few have real economic importance<sup>[34,96]</sup>.

### Rust (Uromyces viciae-fabae (Pers.) Shroet.)

Rust caused by Uromyces viciae fabae pers. Schroet, is one of the most widely distributed diseases of faba bean around the world<sup>[45]</sup>. Rusts attack aerial organs, especially leaves and stems, producing typical red-brownish powdery lesions. It is widespread, but is important only in some humid and warm regions. In general, rust appears late in the season and causes an estimated 20% loss in faba bean production<sup>[7]</sup>. Rust occurs mostly late in the season and therefore, chemical control may not be economical. However, when rust occurs with chocolate spot in the same field, Mancozeb (Dithane-M45) can be used<sup>[72]</sup>. Removal of infected plant debris<sup>[88]</sup>, destruction of other host species and rotating faba bean with non-host crops<sup>[22]</sup>, should play an important role in reducing chances of survival and primary infections in the field.

### Ascochyta (Ascochyta fabae Speg.)

Leaves, stems, and pods exhibit symptoms. On leaves, lesions are dark brown, circular to elliptic, somewhat sunken in the green tissue. The expansion on the leaf changes to an irregular shape and is grayish in the center, with picnidia (a fruiting body containing spores found in certain fungi) becoming apparent. On the stems, spots are similar to those on the leaves but more elon-

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gated. Rotations help in decreasing the importance of the disease.

### Chocolate spot (Botrytis fabae Sardiña)

Losses occurred by chocolate spot forced several faba bean growers to abandon their crops<sup>[39]</sup>. The disease occurs anywhere faba bean is grown. Losses caused by chocolate spot are due mainly to a decreased number of pods per plant<sup>[112]</sup>. However, stem, flower and pod tissues may also become infected. Although stems, pods, and even flowers can show symptoms of chocolate spot under the right conditions, the most affected tissue is the foliar one.

### Mildew (Peronospora viciae (Berk.) Caspary)

Symptoms are visible in leaf margins, which later dry out<sup>[73]</sup>. Crop rotation and destroying crop residuals alleviate the presence of mildew.

### Agronomic management methods

Faba bean is susceptible to several pathogenic fungi. The use of host plant resistance is the best means of disease control, given that management practices are not always effective enough and fungicides are costly (both in economic and environmental terms). Several means are employed to agronomic fungi management in faba beans. Crop rotation is a critical part of control of all of the major diseases and the rotation period needs to take into account the other host species. The use of clean, unblemished and preferably certified seed is vital for minimizing the spread of Ascochyta blight as the pathogen can be carried under the seed coat<sup>[25]</sup>. Reducing relative humidity next to the plant surface is an effective way to hinder the infection process. In addition to their requirements for high humidity and damp leaf surfaces, each of these diseases has an optimum temperature range. Finally, any factor that diminishes plant vigour promotes the development of the disease. Therefore, nutrient deficiencies such as phosphorus or potassium should be avoided, the same as with high weed infestation; equally a good soil drainage has to be maintained, thus preventing water logging. Frost damage also increases susceptibility to chocolate spot<sup>[41]</sup>, so sowing date should be chosen accordingly.

### Chemical and biological methods

Disease control with fungicide is focused on foliar

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sprays. Fungicides are regularly employed to prevent further progress of chocolate spot disease in fields around the world, and have been tested in different studies<sup>[5,79,97]</sup>. Many compounds have been reported as helpful in controlling chocolate spot: benzimidazoles (benomyl, carbendazim), dicarboximides (procymidone, iprodione, vinclozolin), dithiocarbamates (mancozeb), aromatics (chlorothalonil), conazoles (tebuconazole, cyproconazole, metconazole) and strobilurins (azoxystrobin, pyraclastrobin). Chlorothalonil and mancozeb are also recommended for controlling Ascochyta blight<sup>[25]</sup>, and chlorothalonil and carbendazim control Cercospora leaf spot although neither is registered. Mancozeb, chlorothalonil, copper and triadimefon with propineb have been recommended against rust.

### VIRUSES

Up to now, neither viruses nor bacteria have been considered major diseases, except locally in some cases<sup>[17,18]</sup>. No bacterial diseases are worth mentioning<sup>[96]</sup>. Important virus diseases of faba bean are bean yellow mosaic virus (BYMV), bean leafroll virus (BLRV) and broad bean stain virus (BBSV)<sup>[14,101,109]</sup>.

### Bean leafroll virus (BLRV)

*BLRV* is the most important virus known to infect faba bean. It was first isolated from faba bean by Quantz and Volk<sup>[91]</sup> in Germany. The main symptoms produced by the virus are interveinal chlorosis, yellowing, stunting, leaf rolling, reddening and thickening of the leaves, suppression of flowering and pod setting.

### Bean yellow mosaic virus genus potyvirus (BYMV)

*BYMV* was reported in faba bean by Boning<sup>[15]</sup> in Germany. The number of fields infected with *BYMV* can vary greatly among locations. A high incidence, up to 100%, has been noted in some regions of Egypt, Iraq and Sudan. Two of these countries (Egypt and Sudan) are known for their relatively warm winters, which favour increased aphid populations and movement. The effect of *BYMV* on yield depends, to a great extent, on the time of infection and on the strain of the virus.

# Beet western yellows virus genus polerovirus (BWYV)

BWYV was first isolated by Duffus<sup>[30]</sup> in California.

Synonyms for *BWYV* are Malva yellows virus and Turnip mild yellows virus. The main symptoms produced upon infection with BWYV are yellowing and stunting with brown phloem discoloration.

# Broad bean mottle virus genus bromovirus (BBMV)

The symptoms produced in faba bean are, mainly, mottling, marbling or diffuse mosaic, which is often associated with leaf malformation and sometimes with plant stunting. A faba bean loss 35-55%, depending on time of *BBMV* infection has been reported<sup>[67]</sup>. The virus has been found to reach high levels of incidence in faba bean fields in Morocco, Sudan and Tunisia<sup>[32,67,83]</sup>.

# Faba bean nectrotic yellows virus genus nanavirus (FBNYV)

*FBNYV* was first isolated from faba bean near Lattakia, Syria<sup>[56]</sup>. The virus can be very damaging in those years in which an epidemic occurs, as happened in Middle Egypte during 1992, 1997 and 1998 and in Tunisia in 2001<sup>[68]</sup>. One week old faba bean plants show retarded growth as early as five days after inoculation. At two weeks after infection, the plants are usually severely stunted. The leaves become thick and brittle and show interveinal chlorotic blotches, which begin at the leaf margins. Those young leaves remain very small and are cupped upwards, whereas older leaves are rolled downward. New shoots, leaves and flowers develop poorly. About 3-4 weeks after infection, interveinal chlorotic success and flowers develop poorly. About 3-4 weeks after infection, interveinal chlorotic section, interveinal chlorotic section, interveinal chlorotic section.

### Agronomic management methods

There are moderate levels of resistance to viruses in general and particularly for Bean Leaf Roll Virus and Bean Yellow Mosaic Virus but damage is too small to be considered by breeders.

### Chemical and biological methods

Many chemicals have been reported as virus's resistance inducers in plants<sup>[108]</sup>. Among these chemicals, salicylic acid (SA) is considered one of the key components of defence signal transduction, which induces a full set of systemic acquired resistance genes<sup>[36,69]</sup>. SA may have an effect on plant defence mechanisms against harmful diseases. SA can generally control both biotic and abiotic defence programmes<sup>[16,97]</sup>. Radwan et al.<sup>[93]</sup> reported the use of SA to induce resistance in faba bean plants against *BYMV*.

### NEMATODES

Nematodes are serious pests in some regions, although they are also spread in many regions without challenging the yield<sup>[50]</sup>. Stem nematode (*Ditylenchus dipsaci Filipjev*) is the most important to faba beans, especially in cold regions. Young stems thicken, and this thickening later affects the adult stem, which becomes reddish brown at the beginning and ends up almost black. Petioles and leaflets are typically deformed. The infection can pass to the pods and seeds. The parasite can persist in the soil for several years, even without the host presence<sup>[49]</sup>.

### Stem nematode (Ditylenchus dipsaci (Kuhn) Filipjev)

The stem nematode D. dipsaci is a destructive seed and soil-borne pathogen of faba bean in many parts of the temperate region<sup>[38-40,47]</sup>. Infested seeds play an important role in the survival and dissemination<sup>[47]</sup> of the nematode. This is probably why D. dipsaci has a very wide geographical distribution<sup>[44]</sup>. Although several biological races have been reported in stem nematode[102], the 'giant race' is generally more common in the Mediterranean region<sup>[38,40,65]</sup> compared to the 'oat race' in Europe<sup>[44]</sup>. The 'giant race' is responsible for more damage and greater percentage of infested seeds, compared to the 'oat race'[48]. Yield losses as high as 67.8%, with 20% of the seeds infested have been reported from experimental plots, with 650 larvae of the 'giant race' per 100 cc soil, in Syria<sup>[39]</sup>. The first sign of infection is a slight swelling of the young stem and distortion and twisting of petioles and leaves. As the plant matures, the swollen areas on the stem enlarge and turn dark brown to black.

### Agronomic management methods

Chemical treatment of the seeds stained by stem nematode infestation seldom destroys the population, so clean (certified) seed is the best form of prevention. Similarly, transfer of infected straw and plant debris is to be avoided and some nematodes in infected seeds can even survive passage through a monogastric or ruminant mam-

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mal<sup>[85]</sup>. Thus incineration is still considered the most effective method of destroying infected material.

### Chemical and biological methods

Chemical control of nematodes is difficult. The powerful insecticide Aldicarb was considered effective against *Pratylenchus*, *Meloidogyne* and *Ditylenchus* species, but it has been banned within the European Union and in some other regions. Oxamyl is considered effective against root-knot nematodes. Solarization was found effective against several species including *P. thornei* when the temperature under the plastic cover reached  $55^{\circ}C^{[100]}$ .

### PESTS

The insect pests are largely the most important ones<sup>[6]</sup>.

### Bruchus (Bruchus rufimanus Boheman)

The female sets eggs inside the grain at young stages of the pod. The larvae feed inside the seeds, destroying them either when still in the field or later, after they are harvested. The adults overwinter in the granaries and migrate to the field in the spring.

### Sitona (Sitona lineatus L.)

Adults of *sitona* attack the leaves, eating the leaf margins with a set of typical semi circular incisions. Larvae live below the ground, damaging the roots and, especially, the *Rhizobium* nodules that are either destroyed or diminished in their activity<sup>[58]</sup>.

### Lixus (Lixus algirus L.)

*Lixus* are distributed specially in the North Africa and the West Asia. *Lixus* are borers that grow within the stems, drying out the plant at the vegetative stage<sup>[58]</sup>.

### Aphids (Aphis fabae L.)

*Aphids* are polyphagous, especially *Aphis fabae*, a well-known enemy of many plants<sup>[20]</sup>. They are suckers, living in the uppermost parts of stems and in the buds, deforming the plant shape and stopping its growth if not controlled.

### Agronomic management methods

Cultural techniques that include site selection, crop

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rotation, cultivar and seed selection, preferential sowing dates, row spacing and plant density, weed control and more recently stubble retention are all used to reduce insect pest populations<sup>[6,8,54,75,110]</sup>.

### Chemical and biological methods

Pest management in faba beans depend heavily on broadspectrum insecticides, which due to their low cost are often used prophylactically, particularly to control aphids. However strategies to minimize the use of broad-spectrum insecticides are widely used and when required they are where possible substituted with more selective chemicals or bio-pesticides. The dependence of faba bean on bees for pollination necessitates particular care in the choice of chemical and timing of insecticide treatments. Traps utilizing insect sex and aggregation pheromones and feed baits, i.e., poultry mash to attract snails and seed baits for wireworms, are used to monitor pest presence<sup>[10,74]</sup>.

### PARASITIC PLANTS

Weeds in general and the parasitic weeds in particular are major constraints to the faba bean production. Parasites are the most destructive weeds known<sup>[86,99]</sup>. Parasitic weeds adopt different forms to invade host plants. Some invade aerial parts, whereas others invade the underground roots such Orobanche and Striga<sup>[114]</sup>. Parasitic plants vary widely in their degree of host dependence. Parasitic weeds such as Orobanche and Striga are difficult to control because they are closely associated with the host root and are concealed underground for most of their life cycle. The main difficulties that currently limit the development of successful control measures are the ability of the parasite to produce a tremendous number of seeds that may remain viable in the soil for more than 15 years and the intimate physiological interaction of the parasite with host plants. We will now discuss the four most economically significant higher plant parasite groups.

### Orobanche

The broomrape (*Orobanche*) is one of the most important limiting factors in faba bean production throughout the Mediterranean region<sup>[23]</sup> including countries in Europe and North Africa and others in the Near

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East. The damage caused by the parasites *Orobanche* on faba bean field is significant, the main constraint for its cultivation in the Mediterranean area specifically the species *O.crenata*, *O. foetida* and *Phelipanche aegyptiaca* (formerly *O. aegyptiaca*)<sup>[76,86]</sup>.

Broomrapes (*O*. ssp.) are holophrastic weeds that depend wholly on their hosts for nutrition. For instance, *O*. crenata can cause yield reductions of faba bean (*Vicia faba L*.) ranging from 5 to 100 percent depending on the severity of infestation<sup>[86]</sup>. Efficiency of broomrapes, as parasites, pertains to their ability to germinate upon stimulation<sup>[46]</sup>, penetrate into host tissue<sup>[90]</sup>, develop haustorium<sup>[28]</sup>, establish connection through xylem fusion<sup>[23]</sup>, use available food<sup>[113]</sup>, then grow, reproduce, and persist in the soil as dormant seeds.

*Orobanche* is, however, considered an important agricultural parasite in faba bean in Beja region of Tunisia<sup>[60]</sup>. The main *Orobanche* species in Tunisia include *O.crenata, O.foetida* and *O.ramose*<sup>[61]</sup>. The estimated levels of *Orobanche* incidence was indicated that about 5 000 ha out of 70 000ha planted to food legumes might have *Orobanche* infestation and Yield losses are approximate from 20 to 80 percent. *O. foetida* is common in the Northern parts on large and small seeded faba bean beside other species of different families.

Research results indicate that there is a great potential for improving the productivity and yield stability if the biotic and abiotic stress factors are effectively controlled and the inherent yield potential of the cultivars is improved.

### Striga

*Striga* spp. are obligate root hemiparasites and infest an estimated two-thirds of the cereals and legumes in Sub-Saharan Africa<sup>[9,81]</sup>. Several species of *Striga* attack the major cereal crops in Africa (e.g., faba bean, maize, sorghum, millet, and rice). *Striga* constitutes a major biotic constraint to the production of faba bean crop and is considered to be the most devastating parasite in grain production in Africa<sup>[92]</sup>.

### Cuscuta

*Cuscuta spp.* have yellow-to-orange, rootless, leafless vines that attach to the shoots of host plants. They are obligate holoparasites, typically exhibiting broad host ranges, and inflict serious damage to many crops. Seeds of *Cuscuta spp.* have been transported worldwide in contaminated shipments of crop plant seeds. It's commonly known as dodder, are important weeds in Europe, the Middle East, Africa, North and South America<sup>[86]</sup>. *Cuscuta* are obligate parasitic plants with approximately 170 different species throughout the world<sup>[45]</sup>. All species of the genus *Cuscuta* are obligate parasites that attack stems and leaves of a wide variety of host species, including forage crops and vegetables such faba bean.

### Agronomic management methods

There is no single technology to control parasitic plants. The effectiveness of conventional control methods is limited due to numerous factors, in particular the complex nature of the parasites, their tiny and longlived seeds, and the difficulty of diagnosis before the crop is irreversibly damaged. The intimate connection between host and parasite hinders efficient control by herbicides. Prevention is of great importance. On a local level, the sources of infestation can be reduced by controlling the use of contaminated seed lots, or simply by destroying heavily infested crops[58]. Seedbank demise can be efficiently achieved by fumigation or solarization, but this is not economically feasible in relatively low value and low-input crops like faba bean. Some biological control agents have shown promise in managing broomrape, but the technology is not ready yet for commercial application<sup>[3]</sup>. Early plantings of faba bean are often more severely infected so delayed sowing is arguably the best-documented traditional method. Manual weeding is useful just to avoid spreading of the seeds and further increases of the seed bank at the beginning of the infestation in field, but it is not economic in industrialized agriculture. Crop rotation is of limited value due to the long viability of the seeds and the broad host range.

### Chemical and biological methods

Broomrape on faba bean can be effectively controlled by glyphosate, but when the herbicide is applied too early, not enough attachments are controlled. So, broomrape control normally requires lower foliar herbicide application rates than those applied for control of autotrophic weeds. Concerning the biological means, numerous microorganisms that might be useful for biocontrol of broomrape species have been isolated

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and reported in the past, but none has been used widely<sup>[3,13]</sup>. However, the technology is not ready yet for commercial application

### ENGINEERINGAPPROACHES

Significant genetic variation for all these traits of interest exists within numerous faba bean germplasm lines maintained, providing an excellent resource for plant breeders<sup>[29]</sup>. Fast and reliable screening methods have been adjusted to fulfil the needs of breeding programmes both for fungal diseases<sup>[95,106]</sup>, parasitic weeds<sup>[95]</sup> and abiotic stresses<sup>[104]</sup>. Many of these traits of interest have already been incorporated into modern cultivars but several others, many of which are controlled quantitatively by multiple genes, have been more difficult to manipulate. Successful application of biotechnology to resistance breeding in faba bean will require both a good biological knowledge of faba bean and the mechanisms underlying resistance. Although relevant progress has been made in tissue culture and genetic transformation, faba bean is still far from other crops in biotechnological achievements. Similarly, even when significant QTL (Quantitative Trait Loci) studies are being identified<sup>[107]</sup>, this is still insufficient to efficiently apply marker-assisted breeding. The limited saturation of the genomic regions bearing putative QTLs makes it difficult to identify the most tightly linked markers and to determine the accurate position of QTLs. Breeding effectiveness might soon increase with the adoption of the new improvements in marker technology together with the integration of comparative mapping and functional genomics<sup>[27,94]</sup>. Mutagenesis is one of the most critical steps for genetic variation as well as selective breeding. Successful mutant isolation largely relies on the use of efficient mutagens.

### **Chemically induced mutation**

Chemical mutagens are defined as those compounds that increase the frequency of some types of mutations. Chemical mutagens can be divided into two classes those mutagens which can cause mutations to both replicating and non replicating DNA, are called as class I mutagens, those chemicals, which affect replicating nucleic acids are called as class II mutagens.

The molecules of class II mutagens look like nucleic

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acid; hence they are incorporated into the replicating DNA molecule. The most prominent mutagenic members of this class are base analogs and dyes (alkylators).

In plant research, the most popular chemical mutagen, ethylmethane sulfonate (EMS), has been commonly used for this purpose. Although this mutagen can be handled easily and applied to any plant, it primarily produces single base substitutions, but not drastic mutations such as large genomic deletions.

### Physically induced mutation

The ability to induce mutations has been a major driving force in genetics for the past 75 years (Muller 1930). The use of nuclear techniques in plant breeding has been mostly directed for inducing mutations. Since the discovery of X-rays about one hundred years ago, the use of ionizing radiation, such as X-rays, gamma rays and neutrons for inducing variation, has become an established technology. Induced mutations have been used in the improvement of major crops such as wheat, rice, barley, cotton, peanuts, beans, which are seed propagated<sup>[1]</sup>. During the past seventy years, worldwide more than 2250 varieties have been released that have been derived either as direct mutants or from their progenies<sup>[71]</sup>. Induction of mutations with radiation has been the most frequently used method for directly developed mutant varieties. Since the establishment of the Joint FAO/IAEA Division of the Nuclear Techniques in Agriculture, more than 1800 cultivars obtained either as direct mutants or derived from their crosses have been released worldwide in 50 countries<sup>[70]</sup>.

The ionizing radiation (IR) causes a variety of DNA damages, including base and sugar alterations, formation of DNA–DNA and DNA–protein cross-links, as well as single-strand breaks (SSBs) and double-strand breaks (DSBs). It is, however, generally accepted that the DSBs are the main, if not the only type of damage that leads to the cell death<sup>[51]</sup>.

Induced mutations will continue to have an increasing role in creating crop varieties with traits such as modified oil, protein and starch quality, enhanced uptake of specific metals, deeper rooting system, and resistance to drought, diseases and salinity as a major component of the environmentally sustainable agriculture.

Future research on induced mutations would also be important in the functional genomics of many food

# crops. Most mutant varieties were released in China (26.8%), India (11.5%), USSR and Russia (9.3%), the Netherlands (7.8%), USA (5.7%) and Japan (5.3%). Many induced mutants were released directly as new varieties; others were used as parents to derive new varieties. For example, of the 2,252 varieties, 1,585 (70%) were released as direct mutants, i.e. from direct multiplication of a selected mutant and its subsequent release as a new variety. In rice, the majority of mutant varieties were developed as direct mutants selected from mutated populations. The remaining 667 crop varieties were derived through crosses with induced mutants. Mutation induction with radiation was the most frequently used method to develop direct mutant varieties (89%).

### CONCLUSION

In each country or region the faba bean (Vicia faba L.) is grown widely under a range of climatic conditions from temperate to subtropical and it hosts a wide variety of regional, native and exotic cosmopolitan insect pests, fungal pathogens and viruses as well as parasitic weeds so a generalized integrated management strategy is unlikely to be realized. Chemical, agronomic and biological methods developed help in management some payhogens but can not immune the faba bean against all severe conditions and pest. Genetic resistance is available but for some fungi, nematodes and broomrapes, and cultivars with single resistances are not on the market in many countries. High yield and resistance/tolerance to both biotic and abiotic stresses are the prime objectives across faba bean breeding programmes. Mutation breeding using physical mutagens for evolving new genotypes has been used in recent years as a valuable supplement to the method of plant breeding in the development of better crop cultivars<sup>[4,19,42]</sup>.

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