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Abstract

Crop plants are exposed to a plethora of biotic and abiotic stresses. Biotic stresses such as pathogens (viruses, bacteria and fungi), insect pests, nematode parasites and weeds cause a significant loss of crop yield and quality. Although conventional strategies like breeding for resistant varieties and agrochemicals and biocontrol agents for control of diseases and pests have been in use for a long time, these have been met with limited success. During the last 10 years, technological advancements in genetic engineering have led to the development of transgenic crop varieties resistant to various biotic stresses. A large number of transgenic crops have been developed and more are underway; however, the number of biotech crops reaching the field from labs is still limited. Transgenic crops developed against insect resistance and/or herbicide tolerance have been commercial success stories, an example being Bt cotton.

Keywords

Transgenic crops • Biotic stress • Disease resistance • Coat protein • Pathogen-related proteins • Insect resistance • Bt crops • Nematode resistance • Herbicide tolerance • RNAi

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30.1 Introduction

In the present-day scenario, food availability, quality and safety are given top priority, keeping in view the changing climate which will, in the near future, reduce availability of fertile land for agriculture (Easterling et al. 2000) besides directly affecting agricultural yield. Crop yield losses due to biotic stresses like pest infestation, weed overtake and disease occurrence together with abiotic factors like drought, salinity and

extreme temperature diminish considerably food production economics in many parts of the world. With climate change predicted to alter conditions in various parts of the world, researchers started developing models to forecast adverse effects of abiotic and biotic factors on crop productivity. In a study by Collier et al. (2008), it was deduced that changes in life cycle of a pest with increased number of generations or with increase in number of survivors of a particular stage of instar could be a consequence of the changing climatic conditions in the future. Such changes are also predicted for plant pathogens (Lonsdale and Gibbs 2002; Turner 2008). Apart from this, the tremendous increase in human population is bound to elevate food demand in terms of both quality and quantity. In fact, it has been suggested that food production needs to be doubled by 2050 with available arable land to ensure food security for future generations (Baulcombe 2010; Datta 2013). Further, it was estimated that in wheat alone, production needs to increase by 2 % annually (Singh and Trethowan 2007).

Enhancement of food production can be achieved by various strategies: (1) increasing the land area under cultivation, (2) developing improved varieties by traditional breeding methods, (3) adopting better agricultural techniques and tools and (4) adopting transgenic approaches. The first approach is neither feasible nor sustainable, as we can no longer convert scarce forest areas into arable lands. Although conventional breeding has contributed its share, it has limitations such as non-precise gene transfer, transfer of unwanted genes, time-consuming and laborious (Rajam et al. 1998; Bhatnagar-Mathur et al. 2008; Hazarika and Rajam 2011). At the same time, increasing use of land for purposes other than agriculture (like housing, industrial building, forest conservation and so on) also needs to be considered (Evans 2009). In the present context, an effective and challenging task is to maximise land use efficiency by increasing crop productivity per unit area. Therefore, better tools and techniques can only compliment conven-

tional agricultural productivity by use of other novel approaches.

In this regard, transgenic technology has gained relevance for crop improvement and has added a new thrust to agriculture. Better understanding of biotic and abiotic factors involved in crop improvement, and their manipulation for better results, is developing as a potential strategy. Area under cultivation of genetically modified (GM) crops is increasing year by year (James 2013). Although this is a positive sign for increasing acceptance of GM crops in the commercial market (with a notion that the productivity of these crop varieties can deal with the demand for quantity and quality of food) (Chakraborty et al. 2000; Datta 2012), concerns regarding the effects of these GM products on human health do persist (Dona and Arvanitoyannis 2009). It is to be noted here that in both conventional breeding techniques and transgenic crops, change in the genetic make-up of the plant may be involved. However, in the production of GM varieties via the transgenic technology, genetic changes that are brought about are small and defined, whereas in conventional breeding, these changes can be relatively large and may involve a set of ill-defined, uncharacterised genes (Datta 2013). In this chapter, we have given a general overview of biotic factors and transgenic approaches being used for alleviating these stresses for improving crop health and productivity.

30.2 Types of Biotic Stress

Plant species are under a constant threat from a number of biotic stresses that exist simultaneously in nature. Attack by pathogens and parasites such as bacteria, fungi, viruses and nematodes plays a crucial role in plant growth and productivity. Apart from these, insect herbivory and theft of nutrients by weeds also cause serious economic losses in crop yield. These stresses very often coexist, leading to multiple stresses which might elevate the level of biotic stress incident on crop plants.

30.3 Conventional Strategies to Alleviate Biotic Stresses

To protect plants against biotic stresses, various prevention and control strategies have been tested over the ages. Conventional breeding practices for the generation of disease-resistant lines against diverse biotic stresses in plants have been widely employed over several decades now. Besides conventional agricultural practices, farmers depend on chemical pesticides and herbicides to a great extent which leads to significant increase in crop yields. However, the ever-increasing environmental and health concerns against use of chemicals in agriculture paved the way for the development of biological control agents (BCAs). Biological control measures include use of BCAs, i.e. use of antagonists that suppress plant diseases; use of predatory insects, entomopathogenic fungi, bacteria and nematodes for control of insect pests; and use of weed-specific pathogens for control of populations of specific weeds. Formulated natural plant products are also used in integrated pest management (IPM) strategies.

30.4 Why the Need for an Alternative?

Although BCAs and resistant lines of crop plants are successfully used for some plant disease and insect pest control, emergence of resistance-breaking biotypes is rapid in pace. Conventional plant breeding techniques are laborious and time-consuming. To keep pace with the emergence of resistant biotypes of pests, breeders need to look for new resistant traits that are more durable. However, keeping the time required for developing a new cultivar in view, it becomes difficult to rely wholly on such a strategy. Advancements in the field of genetic engineering and molecular biology over the past two decades have led to the use of transgenic technology in crop improvement.

30.5 Transgenic Approaches to Biotic Stress Tolerance in Plants

The high potential of genetic engineering in developing disease-resistant transgenic crop plants has been successfully tapped as a strategy alternative to conventional breeding. Over the years, success stories have thrown up transgenic plants resistant to various plant pathogens and insect pests, and these engineered crops have moved to the field (James 2013) instead of remaining as just laboratory curiosities. Thus, transgenic crop plants engineered for resistance against bacteria, fungi (Wally and Punja 2010), virus (Prins et al. 2008), pathogens and nematodes (Atkinson et al. 2003; Tamilarasan and Rajam 2013) have become a reality, so also resistance against insect herbivory (Christou et al. 2006; Gatehouse 2008) and for dealing with the unwanted weeds (Herouet-Guichenev et al. 2009).

30.5.1 Viruses

Plant viruses cause significant losses in crop yield and productivity on a global scale. The most devastating infectious plant diseases are caused by viruses (Anderson et al. 2004) belonging to the *Tospovirus* group, causing crop losses of more than a billion dollar a year (Prins and Goldbach 1998). In view of the limited effectiveness of control mechanisms like the use of virus-free seeds, prevention by phytosanitation, breeding for resistant lines or prevention of vector transmission (Mawassi and Gera 2012), the transgenic approach has been used. Introduction of natural virus-resistant genes into an existing, desirable cultivar can be achieved using genetic engineering. Pathogen-derived resistance (PDR) is the most commonly used phenomenon. Here, transgenic plants expressing genes of a viral pathogen show resistance against the same or related viruses (Slater et al. 2003). The first

application of PDR phenomenon with respect to plant viruses was demonstrated by Powell-Abel et al. (1986) who showed that transgenic tobacco plants expressing tobacco mosaic virus coat protein gene were resistant to attack by the tobacco mosaic virus. Examples of pathogen-derived genes include the replicase protein (Rubino and Russo 1995), coat protein (Lindbo and Dougherty 1992; Nelson et al. 1993; Ganesan et al. 2009), proteinase (Vardi et al. 1993), movement protein (Cooper et al. 1995) or genes coding for host cell lethal proteins like antiviral compounds (ribosome-inhibiting proteins) (Wang et al. 1998) or those that target the viral genome or particle directly, viz., ribozymes and ribonucleases (Sano et al. 1997). Resistance against viruses can also be obtained by presetting the RNA silencing system in plants by introducing and expressing fragments of sense and anti-sense viral nucleic acid sequences into chromosomes of the plants (Prins et al. 2008; Tyagi et al. 2008; Duan et al. 2012), or by expressing the virus mini-replicon (Brumin et al. 2009), or by expression of artificial microRNA (Niu et al. 2006). Management of papaya ringspot virus (PRSV) is one of the remarkable success stories (Gonsalves et al. 2004).

30.5.2 Bacterial and Fungal Pathogens

Research over the years has led to the use of various strategies for the development of transgenic plants resistant to bacterial and fungal pathogenic attack (Salomon and Sessa 2012), the first among these being expression of resistance genes (R-genes). R-genes code for nucleotide-binding peptides harbouring leucine-rich repeats (LRRs) which are transmembrane in nature (Bent and Mackey 2007). These peptides recognise products of avirulence (*Avr*) genes produced by the pathogen and subsequently induce activation of diverse downstream processes that include activation of pathogenesis-related genes (PR genes), production of high amounts of inhibitory molecules and their accumulation and induction of the hypersensitive response (Kiralý et al. 2007).

Introduction of R-gene *Rxo1* from maize into rice led to the latter's resistance to bacterial streak disease caused by *Xanthomonas oryzae* pv. *oryzicola* (Zhao et al. 2005a), while expression of R-gene (RPI-BLB2) from wild potato conferred resistance to the cultivated potato against attack by *Phytophthora infestans* (van der Vossen et al. 2005).

Many fungal pathogens produce toxins called mycotoxins in general. *Fusarium culmorum* and *F. graminearum* produce deoxynivalenol, a trichothecene mycotoxin, which confers virulence to these fungal strains and also poses toxicity threat to humans and animals (Kimura et al. 2006). Looking into the way in which the fungus deals with the mycotoxin, researchers developed transgenic lines of wheat, rice and barley. These genetically modified (GM) plants overexpressed the trichothecene 3-O-acetyltransferase (*tri101*) gene, whose product is involved in acetylating deoxynivalenol which results in a much less active form of the toxin (Manoharan et al. 2006). The transgenic wheat line showed reduced wheat spike infection (Okubara et al. 2002), and transgenic rice (Ohsato et al. 2007) and barley (Manoharan et al. 2006) yielded grains with far less mycotoxin contamination than in the wild type.

Use of antimicrobial peptides and PR-proteins in engineering plants for resistance against pathogens is the most common strategy. These proteins are expressed constitutively in plants; however, their levels shoot up upon pathogen attack (Rao et al. 1999; Wally and Punja 2010). Antimicrobial metabolites thus produced are involved in degrading fungal components like the cell wall, cell membrane or RNA, or these induce secondary metabolite production or increase physical barriers in a plant cell (Ferreira et al. 2007). A wide range of such peptides have been overexpressed in plants for conferring resistance against different plant pathogens, to name a few, chitinases, β 1-3 glucanases, defensins, thionins, thaumatin-like proteins (Rajam et al. 2007), lipid-transfer proteins and phenylalanine ammonia lyase (reviewed by Wally and Punja 2010). Recent reports show that introduction of alfalfa glucanase into eggplant led to increased resistance

against pathogenic fungi like *Verticillium dahlia* and *Fusarium oxysporum* (Singh et al. 2014).

Interestingly, polyamines like diamine putrescine, triamine spermidine and tetra-amine spermine have been implicated in abiotic (Rajam et al. 1998) and biotic (Hazarika and Rajam 2011) stress tolerance (Hussain et al. 2011) besides their involvement in the regulation of a wide variety of biological processes including cell division, growth and development, stabilisation of DNA and gene regulation (Igarashi and Kashiwagi 2010). Although considerable amount of work has been done on genetic manipulation of polyamine biosynthesis genes for abiotic stress tolerance in various plants (Marco et al. 2012), engineering polyamine biosynthesis for biotic stress tolerance has not been explored much so far, as evidenced by scarce literature on the subject. For instance, enhanced fungal resistance was reported by overexpressing the polyamine biosynthesis gene, S-adenosylmethionine decarboxylase, in tobacco (Waie and Rajam 2003) and tomato (Hazarika and Rajam 2011) and arginine decarboxylase in eggplant (Prabhavathi and Rajam 2007).

RNA interference (RNAi) strategy involving plant-mediated silencing of fungal genes shows promise for developing potential tools in the fight against fungal attack on crop plants in the near future. Fungal uptake of synthetic siRNA specific to the ornithine decarboxylase (*ODC*) gene led to downregulation of *ODC* gene and in turn caused significant decrease in mycelial growth and sporulation in the fungal pathogen, *Aspergillus nidulans* (Khatri and Rajam 2007). Transgenic tobacco plants that expressed antisense RNA of heterologous (mouse) ornithine decarboxylase gene were demonstrated to be more resistant to *Verticillium* wilt compared to the wild type plants (Rajam 2012a). Also, development of powdery mildew causing fungus in wheat and barley, *Blumeria graminis*, was affected adversely by *in planta* expression of antisense transcripts and dsRNA specific to the fungus (Nowara et al. 2010). These initial steps might pave the way for the development of a promising tool for control of fungal plant pathogens (Rajam 2012b). However, success of transgenic crops showing

resistance to bacterial and fungal pathogens is limited in comparison to insect-resistant transgenic crops (Punja 2006).

30.5.3 Insect Pests

Success story of the transgenic plants for insect resistance started with transforming plants with *cry* genes from *Bacillus thuringiensis* (Vaeck et al. 1987). These genes code for highly insecticidal crystal proteins called Bt toxins. Upon ingestion, the toxin binds to specific receptors in the insect midgut epithelium, and further action of the proteases activates the toxin. This active toxin induces lytic pore formation in the midgut epithelial cells, leading to their lysis, and subsequently kills the insect larva (Daniel et al. 2000). Over 400 genes coding for these toxin variants have been identified from different strains of *B. thuringiensis* (Crickmore et al. 2007). Insecticidal spectrum of each of these toxin variants is distinct. Since the first transgenic tobacco and tomato plants were developed carrying *cry* genes (Vaeck et al. 1987), tissue-specific or constitutive expression of Bt toxins has been established in many crop plants against specific pest species (Pierpoint and Hughes 1996). Bt cotton is one among them and is a commercial success. Apart from Bt toxin, *B. thuringiensis* also expresses other proteins like vegetative insecticidal protein (Vip) which is, obviously, insecticidal in nature. Ingestion of Vip proteins leads to the swelling and osmotic lysis of midgut epithelial cells, causing death of the target insect. Unlike *cry* toxins, these are expressed both during vegetative growth and sporulation of the bacterium (Estruch et al. 1996; Crickmore et al. 2007). Like Bt, different Vip toxins act against specific groups of insects. Although Vip3 showed very high insecticidal activity against major lepidopteran pests attacking maize and cotton (Estruch et al. 1996; Fang et al. 2007), licence granted for release of GM Vip cotton is limited and controlled (Crothers 2006). Other entomopathogenic bacteria like *Photobacterium luminescens* also express insecticidal toxin complexes whose genes have been introduced into plants

(Liu et al. 2003). Transgenic *Arabidopsis thaliana* plants expressing *tcdA* gene encoding for toxin A showed resistance against tobacco hornworm and southern corn rootworm.

Apart from genes from microbes, genes from the insect itself are used for killing the pest. One such gene is chitinase, which is a development-associated gene in insects and is expressed during larval moulting, i.e. shedding of the peritrophic membrane and exoskeleton. Insect pests feeding on transgenic plants constitutively expressing insect chitinase are exposed to a constant level of this enzyme all through their development cycle, which disturbs their process of regular moulting. Ding et al. (1998) showed that transgenic tobacco plants expressing chitinase conferred resistance to lepidopteran pests.

Plant transformation with plant genes encoding for insecticidal secondary metabolites like proteinase inhibitors, alpha-amylase and lectins has also been shown to confer resistance to insect attack. In insects, proteinases are among the digestive enzymes essential for catalytic release of amino acids from the ingested proteins which, thereby, contribute to the insect's growth and development. Hence, ingestion of proteinase inhibitors could prove lethal to insects. Proteinase inhibitors are produced in high amounts and are accumulated by plants in response to herbivorous attack or mechanical injury (Ryan 1990). Although plants expressing different proteinase inhibitors, viz., BTI-CMe (barley trypsin inhibitor) and MTI-2 (mustard trypsin inhibitor), showed significant insect resistance (Altpeter et al. 1999; De Leo et al. 2001), genes for proteinase inhibitors like cowpea trypsin inhibitor (CpTI) have been used in combination with Bt toxins to enhance protection of the transgenic crop against insects (Gatehouse 2011).

Expression of protein inhibitors of proteases (Wolfson and Murdock 1987; Thomas et al. 1995) in plants had a significant effect on reproductive output of target insect pests. Exploiting the neuropeptides and inhibitors of polyamine biosynthesis with potential insecticidal activity might prove beneficial for developing insect resistance in plants (Rajam 1991; Raina et al. 1994; Kumar et al. 2009).

With ever-increasing knowledge in the field of RNAi, RNA-mediated (sense or antisense RNA, dsRNA, artificial microRNA) strategies are being employed to induce RNAi in transgenic plants as an effective defence mechanism against insect attack (Mao et al. 2007; Baum et al. 2007; Terenius et al. 2011). One of the major achievements is the use of this strategy to control sap-sucking insects like aphids. Recent success with the use of plant-generated dsRNA and other small RNA-mediated approaches, for conferring resistance against aphids, is remarkable (Pitino et al. 2011; Guo et al. 2014).

30.5.4 Nematode Parasites

Plant parasitic nematodes can cause crop yield losses up to 20 % in a single crop, and in most cases, these losses are caused by attack of root-knot nematodes and cyst nematodes (Koenning et al. 1999; Bird and Kaloshian 2003; Tamilarasan and Rajam 2013). Conventional strategies like crop rotation do not hold good for control of nematodes with a wide host range, and chemical practices are expensive (Abad et al. 2003). Alternate transgenic approaches and RNAi, which was in fact discovered in the nematode *Caenorhabditis elegans* (Fire et al. 1998), provide a better scope for control of plant parasitic nematodes. Some of the strategies used for developing transgenic plants with resistance to nematodes include anti-invasion and migration, anti-nematode feeding and development and feeding-cell attenuation strategies (Atkinson et al. 2003). Expression of cysteine proteinase inhibitor (cystatin) in transgenic plants helped potato plants resist nematode attack (Cowgill et al. 2002). The parasitism genes express parasitism proteins which help the nematode infect the root and parasitise the plants (Davis et al. 2004). Using RNAi approach, *Arabidopsis* plants were bioengineered to express dsRNA of one of the parasitism genes, *16D10*. These transgenic plants were resistant to four species of root-knot nematodes (Huang et al. 2006). Therefore, this strategy can be applied to agriculturally important crops for developing resistance against

nematodes. Similarly, the nematode genes involved in its development and reproduction and the nematode-responsive host plant genes have been targeted for developing effective methods of plant nematode control (for review, Tamilarasan and Rajam 2013).

30.5.5 Weeds

Herbicides are chemicals which are used to kill the weeds, but at the same time, they are deadly to the cultivated crop plants also. Hence, development of transgenic plants resistant to herbicides was taken up. One of the most commonly used herbicide, glyphosate, inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase. Overexpression of this enzyme in transformed *Petunia* plants conferred resistance to this herbicide (Shah et al. 1986). Expression of an insensitive form of EPSP synthase also showed similar results (Herouet-Guicheny et al. 2009). Since then, this strategy has been used for several crop plants. Glyphosate-tolerant soybean was successfully commercialised in 1996, and later, GM crops like herbicide-tolerant maize, cotton, etc. were also accepted by the farmers (James 2013). Recent studies showed that transformation with two genes, glyphosate acetyl transferase *gat* and EPSPS *G2-aroA* genes, conferred high level of tolerance towards glyphosate in tobacco plants (Din et al. 2014). Further, stacking of genes for herbicide tolerance and insect resistance has also been reported in crops like cotton, and these are being commercialised (James 2013).

30.6 Concerns About Transgenic Plants and Novel Alternative Strategies

In spite of the various advantages of the use of transgenic technology, there are some limitations. One major concern, in general, about the release of transgenic plants into the natural environment is transgene flow. Gene flow is a natural process among related species (vertical gene

flow) or related genera within a family (diagonal gene flow) or, even, among unrelated species (horizontal gene flow). However, transgenes that code for traits not found in related species, or even genera, would not integrate into these plants had there not been a transgenic. This unnatural event might pose a threat to the natural gene pool over time (Gressel 2012). The most common cases reported in this context are flow of herbicide-resistant genes from crops to weeds as reported in transgenic glufosinate-resistant rice (Song et al. 2011). The ability to control weeds in such cases, thus, becomes close to nil. Various techniques for transgene containment to prevent such transgene flow have been discussed recently (Gressel 2012). In the case of a leaky containment mechanism where such a transgene movement takes place, methods of transgene mitigation to suppress its spread and establishment in the population have also been highlighted (Gressel 1999, 2012).

Development of resistant pest populations is another shortcoming of transgenic crops expressing insecticidal genes. This has been well noted in Bt crops (Shelton et al. 2003; Moar et al. 2008). Although development of resistant target pest populations against Bt crops has been slower than against that of Bt sprays (Fox 2003; Gressel 2012), there are field reports of Bt-resistant pest populations, as observed in *Spodoptera frugiperda* and *Helicoverpa zea* (Moar et al. 2008; Tabashnik et al. 2008). To this problem, 'refugia' have worked as a solution. Planting regular rows of Bt and non-Bt crops allows survival of the susceptible pests. Bt-resistant trait is recessive, and as long as the susceptible mating population is within reach, selection of resistant genes in the population is expected to be very meagre (Bates et al. 2005). Pyramiding of two or more *cry* genes, or different insecticidal genes, is expected to delay eruption of resistant pest species, as the pest needs to then evolve resistance against two or more insecticidal toxins simultaneously (Zhao et al. 2005b; Watkins et al. 2012).

A more recent advancement in this field is the RNAi approach, which is an alternative to expression of transgenes and might prove to be advantageous over the latter, considering the

limitations and concerns of the transgenic approach. RNAi technology can be applied for control of those insect pests where effective Bt genes are not available, e.g. sucking pests like aphids and jassids. One other important point is its species specificity which reduces the risk of negative effects on nontarget organisms (Huesing et al. 2009). Unlike the existing transgenic crops which are based mainly on transgene protein, RNAi strategy works at the post-transcriptional level where there is no protein in the transgenic plant, and therefore, it is very unlikely that the target pathogen or pest would gain resistance in the absence of the transgene protein (Rajam 2011; Rajam and Singh 2011). A case in point is Bt crops and other transgenic plants where target pathogens or pests have been shown to be resistant after a few generations (Moar et al. 2008; Rajam and Singh 2011). Further, a major concern with transgenic plants is the possible allergic response in the consumers against the expressed transgene protein (Niblett and Bailey 2012) which, on the other hand, in RNAi plants, might not be the case due to absence of any transgene protein. This makes these RNAi plants and their products safe for human and animal consumption and greatly reduces the regulatory issues related with their commercialisation (Auer and Frederick 2009). The beauty of RNAi approach is that more than one gene can be effectively targeted using chimeric RNAi constructs (Gupta et al. 2013; Sinha and Rajam 2013) to the pathogen or pest by this technique. This would also avoid development of resistance by target organisms and would allow targeting of more than one pathogen or pest, as chimeric RNAi construct contains sequences of more than one target gene (Rajam 2011; Rajam and Singh 2011). However, RNAi approach has at least one limitation, i.e. the sequence(s) introduced can have off-target effects on the host plant or other organisms, depending upon the extent of homology between the introduced sequence and the sequence of nontarget genes (Du et al. 2005).

Details on the use of RNAi technology for crop improvement, including control of diseases and pests, are available in the following chapter.

30.7 Conclusions and Future Prospects

The most important point which needs consideration in the use of transgenic approach for alleviating biotic stress in plants is stability of the resistance in engineered plants. Although a large number of transgenic plants resistant to various biotic stresses are under development, only a few have become commercially successful. Examples of such commercial successes are Bt crops. One of the main features responsible for acceptance of Bt crops in the commercial market by consumers was the proof provided for Bt toxin as having no effect on birds and mammals, including humans (Goldberg and Tjaden 1990; Mendelsohn et al. 2003). For large-scale public acceptance of transgenic technologies, the transgenics need to be of proven safety for human and animal consumption; also, the economic balance, i.e. cost involved in developing, raising and registering the transgenic crops, should be balanced with profit margins of their producers, and finally, ease of their availability to the consumer must be ensured.

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