



## Genomic assisted breeding and holistic management of abiotic and biotic stress in silkworm host cultivation: A review

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### ABSTRACT

Silk is a high-value, low-volume product, produced by an important insect commonly known as the silkworm. Sericulture serves as a source of livelihood for farmers besides being an important source of economy for many countries including India. Sustainable production of premium silk depends on continuous production of quality foliage as feed for silkworms obtained from host plants. The production of silk is significantly hampered when host plants are subjected to biotic and abiotic stresses. The foliage harvest could be enhanced when these constraints are efficiently managed by the development of stress-resistant host cultivars. Improved stress-resistant cultivars have been developed using conventional breeding strategies and used in commercial cultivation. However, the highly heterozygous genetic nature of the hosts makes it difficult to understand the inheritance and expression of these quantitative traits. Adoption of appropriate conventional breeding strategies along with genomics tools such as genome-wide association studies, transcriptomics, proteomics, metabolomics and advanced OMICS approaches could prove handy in the development of improved and stress-resistant cultivars. Deeper understanding of the mechanism of tolerance to various stress is required in breeding for improved cultivars. The number of stress-tolerant cultivars is scanty and therefore, holistic management of these stresses through an inter-disciplinary approach could be the most suitable strategy. Adoption of appropriate cultural practices and control measures is necessary for sustainable production under stress regimes. This comprehensive review holds great importance in improving silkworm host cultivation and to researchers in the field of sericulture.

**Keywords:** Abiotic stress, Breeding, Biotic stress, Genomics, Sericulture, Silk

Sericulture is an important agro-based industry in about 70 countries, among which China, India, Vietnam, Uzbekistan, Brazil, Thailand and Bangladesh are the leading silk producing countries. Sericulture provides most employments to farmers next to agriculture in India (Vijayan 2009). Total export earnings from silk goods during 2021–22 was ₹1,848.96 crores (CSB annual report 2021–22). There are two types of silk fibres namely, mulberry silk from *Bombyx mori* (L.) and Vanya silk or non-mulberry silk such as tasar silk, eri silk and muga silk produced from *Anthereae mylitta* Drury, *Philosamia ricini* and *Anthereae assamensis*, respectively. India produces both types of silk but a major proportion share comes from mulberry silk. Cultivation of host plants is a costly affair as the tree is perennial with a long gestation period requiring large space for individual plants. Major cost of mulberry silk production is incurred in mulberry cultivation. The decreasing ground water

availability affects the crop production and could particularly affects livelihood of small-scale farmers (Zhiipao *et al.* 2023). Thus, the economy of sericulture heavily depends on cost-efficient cultivation of hosts to achieve good foliage yield to increase silk (Vijayan *et al.* 2012).

Though mulberry can grow in a wide range of climatic conditions, foliage production is hampered by several biotic and abiotic stresses (Shukla *et al.* 2019). The effects of these stresses on mulberry vary depending on the severity and duration of the stress, as well as the stage of growth and physiological condition of the plant. Various foliar diseases not only reduce leaf productivity (10–20%) but also affects its quality (Khurana and Checker 2011). Drought and salinity are the most devastating abiotic stresses as about 50% of mulberry acreage belongs to arid and semi-arid regions (Khurana and Checker 2011). It is important to adopt suitable chemical and agronomic management practices to control these stresses to utilize the potential land surfaces. However, the use of chemical is not sustainable as it affects the quality of leaves, larval growth and development, and quality of the cocoon (Adolkar *et al.* 2007). Development of superior cultivars under stress regimes assume great importance for the sustenance and productivity of sericulture. Hence, use of

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appropriate plant breeding methods and advanced breeding tools are crucial to develop stress resilient host varieties. The use of stress resistant cultivars and adoption of suitable agronomics practices and other control measures such as physical, chemicals, biological and suitable management of stress would be economical for sustainable production. The objective of this review is to provide an overview of stress breeding in silkworm hosts and suggest adoptable breeding strategies. The review also highlights various management strategies and provides comprehensive information on management of both abiotic and biotic (disease) stresses in host cultivation.

#### Stress breeding strategies in silkworm hosts

Mulberry cultivars with improved leaf yield and higher quality with wider adaptability to abiotic stresses such as drought, salinity, alkalinity, cold and resistance to diseases have been developed using conventional breeding methods (Mogili *et al.* 2023). Abiotic stresses particularly drought and heat are quantitative traits and the underpinning mechanisms are more complex than other traits such as biotic stresses which are generally characterized by monogenic resistance (Lamaoui *et al.* 2018). Considerable efforts have been directed towards breeding for salinity tolerance (Vijayan *et al.* 2009) but presently cultivars suitable for alkaline, saline and acidic soil (Tikader and Kamble 2007) are few. Various strategies have been adopted to improve silkworm hosts. Graphical representation of the hypothetical breeding strategies for host plant improvement is given in Fig 1.

#### Conventional breeding strategies

**Pre-breeding:** Pre-breeding mainly deals with the germplasm enhancement of genetic variability of the genetic base and the use of the improved or pre-bred materials in cultivar development through conventional breeding (Sharma *et al.* 2013). Current breeding programs in mulberry are based mostly on cultivated species, *Morus alba* and *M. indica*, and there is a need to integrate exotic/wild species such as *M. laevigata* and *M. serrata*, with desirable traits into the stress breeding programs (Tikader and Dandin 2007). Use of exotic lines provides promising scopes to introgress characters such as drought, frost and disease resistance into existing elite varieties (Tikader and Kamble 2008).

Efforts have been invested in the collection, conservation and introduction of mulberry genetic resources (Tikader *et al.* 2010).

**Characterization of germplasms:** Characterization of germplasm accessions is essential for the selection of suitable parents for hybridisation or clonal propagation. Saeed *et al.* (2016) identified through transcriptomics, 24,049 Simple Sequence Repeats (SSRs), 1,201,326 (Single Nucleotide Polymorphisms) SNPs and 67,875 (Insertion and Deletions) InDels in wildily growing mulberry species which could be useful in identification of important genes and characterization of germplasms. Inter Simple Sequence Repeats (ISSRs) are the most commonly used markers in mulberry for deciphering the genetic diversity (Khurana and Checker 2011). Marker systems such as Randomly Amplified Polymorphic DNA (RAPD) and Directed Amplification of Minisatellite DNA (DAMD) (Bhattacharya and Ranade 2001), SSRs and AFLPS for charcoal rot resistance (Pinto *et al.* 2018), SSRs for root-knot nematode resistance (Arunakumar *et al.* 2021) were used for germplasm characterisation. SNPs have begun to claim popularity among breeders due to higher density and uniform distribution across the genome (Vijayan *et al.* 2022). Phenotypic characterization was done for resistance to bacterial (Banerjee *et al.* 2009) and powdery mildew (Chattopadhyay *et al.* 2010) among the germplasm lines.

**Hybridization of selected parents:** Interspecific hybridization between red mulberry (*Morus rubra* L.) and white mulberry (*Morus alba* L.) revealed that the magnitude and direction of introgression is asymmetrical, bidirectional and related to the frequency of the parental taxa (Burgess *et*

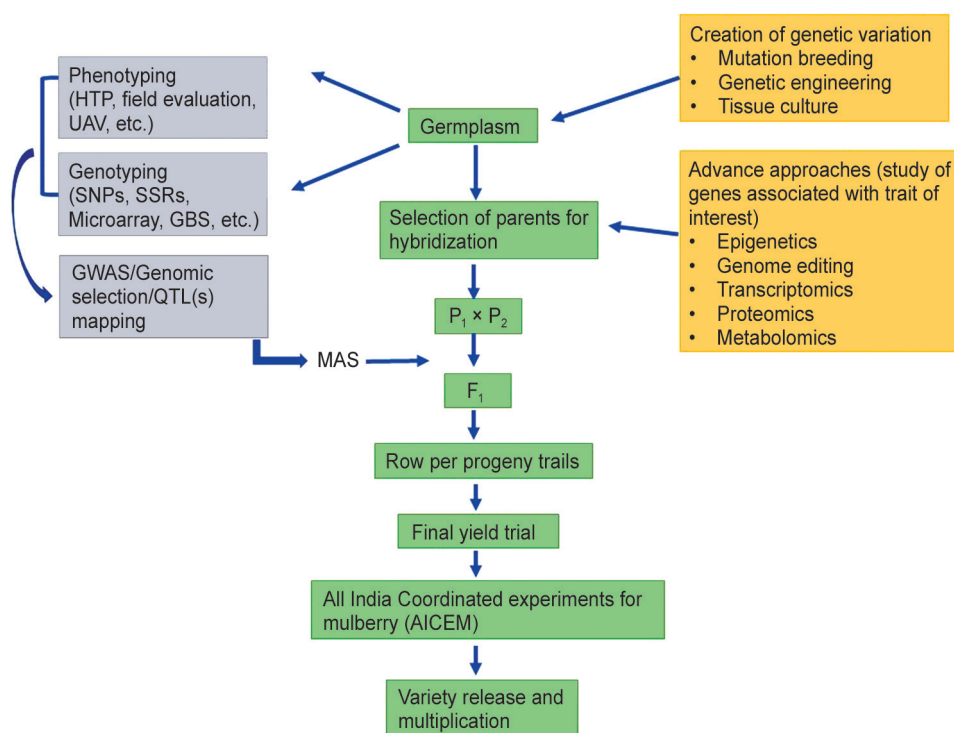


Fig 1 Graphical display of breeding activities involved in the improvement of silkworm host for biotic and abiotic stress tolerance.

al. 2005). Seed setting percentage ranged from moderate to high when cultivated species, *Morus indica* was crossed with wild species, *M. cathayana* (39.5%), *M. tiliifolia* (41.11%), *M. serrata* (47.6%) and *M. pendulata* (75%) (Tikader and Anand Rao 2002). Silkworm host breeding primarily depends on F1 hybrid owing to its highly heterozygous nature and long gestation period (Vijayan 2010). Controlled crossing experiment led to the identification of cold tolerant hybrids such as CT-44 and CT-11 which out yield check variety S1635, by 17.17% and 7.11%, respectively (Doss *et al.* 2012). Genotypes with enhanced water use efficiency have been developed through Dudia White × MS3 cross (Mishra 2014). Hybrids can also be developed by exploring presence of male sterile lines in mulberry and other hosts. In other crops such as maize Pal *et al.* (2020) could develop hybrids by using male sterility system present in the crop.

**Polyploid breeding:** Different ploidy levels are reported in silkworm host plants. Leaf size, fruit size and breast-height diameter are higher in autotriploids than diploids (Anju *et al.* 2021). In a recent study, the triploid cultivar, Shaansang-305 was reported to adapt better to drought stress than diploid Shinichinose (Liu *et al.* 2021). Polyploids with higher chromosome numbers thrives better at higher altitudes. However, tetraploids are less preferred due to coarser leaf, more trichomes and lesser leaf succulence.

**Genetic engineering:** Host improvement could be achieved through genetic engineering approach particularly *Agrobacterium*-mediated transformation. Overexpression of HVA1, a group-3 LEA protein in transgenic mulberry confers salinity, drought (Lal *et al.* 2008) and cold tolerance (Checker *et al.* 2012). Transgenic mulberry expressing the osmotin gene driven by *rd29A* promoter, exhibited drought and salinity tolerance with enhanced membrane stability, osmolytes accumulation and higher photosynthates (Das *et al.* 2011). Overexpression of miR166f was found to enhance drought tolerance in transient transgenic mulberry (Li *et al.* 2018). Previous studies have identified genes controlling abiotic stress tolerance; *MmSK* (Shaggy-like protein kinase) gene as drought stress regulator (Li *et al.* 2018); *MuGABA-T* gene regulates GABA (Gamma-aminobutyric acid) catabolism and induce salt stress tolerance (Zhang *et al.* 2022). The list of genes studied in association with some abiotic stress in mulberry is given in Table 1.

#### Advance omics approaches

Omics approaches such as genomics, transcriptomics, genome editing, epigenomics and marker-assisted selection (MAS) will be rewarding in sericulture. MAS was reported to be an efficient strategy in broadening the genetic base and development of hybrids (Duo *et al.* 2021). MAS is highly beneficial to breeders as it reduces time as compared to traditional approaches, early selection in seedling stage, reduces linkage drag and improvement of traits with low heritability (Hossain *et al.* 2022). One prospect of silkworm host breeding is leaf nutrient quality improvement. Crop biofortification was considered as the most economical, sustainable strategy (Hossian *et al.* 2023). Accessible Morus Genome Database (MorusDB) provides opportunities for genomics studies. The advent of molecular cloning and sequencing coupled with gene transfer has opened new paths for developing the salt-tolerant plants (Vijayan 2009). Reference genes for different traits were reported: *Actin* (ACT3) for drought stress, *PP2A* (*Protein Phosphatase 2A*) for salt stress, *RPL3* (*60S ribosomal protein L3*) for heat stress and *Ubiquitin* (Ubiquitin-like protein 5) for cold stress (Shukla *et al.* 2019).

**Transcriptomics:** To date several EST projects have been completed in mulberry generating a wealth of information. Studies on genes related to various stresses such LEA, RD22, dehydrins and Hal3 and membrane transporters (vacuolar Na<sup>+</sup>/H<sup>+</sup> antiporter and aquaporins) provide the base to adopt different strategies for genetic improvement of silkworm hosts (Khurana and Checker 2011). *MIChi* gene family was found to be the candidate genes responsible for stem rot resistance in mulberry (Jiang *et al.* 2020). Likewise, Li *et al.* (2022) revealed that *MaWRKYIII8* gene is associated with response to drought in mulberry.

**Proteomics and metabolomics:** Although the use of proteomics and metabolomics in mulberry is currently in infancy, these promising tools could be exploited to develop desired biotic and abiotic stress-resilient cultivars. Wang *et al.* (2023) employed Tandem Mass Tag (TMT)-based proteomic profiling methods and studied the effects of salt stress in germinating seeds of *Morus alba* L. and reported that primarily the rate of germination and radicle length were affected by salt stress. On the other hand, metabolomics can decode the biochemical functions of genes and the

Table 1 List of genes associated with abiotic stress tolerance in mulberry

Gene	Gene description	Abiotic stress	Reference
<i>WAP21</i>	Water allocation plan	cold tolerance	Ukaji <i>et al.</i> (1999)
<i>COR</i>	Cold on regulation	cold tolerance	Ukaji <i>et al.</i> (2001)
<i>SHN 1</i>	Schnurri from <i>Drosophila melanogaster</i> ;	drought tolerance	Aharoni <i>et al.</i> (2004)
<i>HVA1</i>	<i>Hevea braziliensis</i> abiotic stress gene	drought and salinity stress	Lal <i>et al.</i> (2008)
<i>bch</i>	L inhibitor 2-aminobicyclo- (2,2,1)-heptane-2-carboxylic acid	drought and salinity stress	Khurana (2010)
<i>NHX</i>	Na <sup>+</sup> /H <sup>+</sup> exchanger	drought and salinity stress	Khurana (2010)
<i>Osmotin</i>	Osmotic stress induced gene	drought and salinity stress	Das <i>et al.</i> (2011)

Source: Vijayan *et al.* (2011)

interaction of such genes in plant metabolism. Significant genotypic variance was reported in the expression pattern of *MiSMT* (*Morus indica*, Sterol Methyl Transferase) and *MiVR* (*Morus indica*, Vestitone reductase) under control and salt stress conditions (Tetorya 2010).

#### *Agronomic management of important abiotic stresses in silkworm host plants cultivation*

**Management of acidic soil:** Mulberry plants are more or less tolerant to acidic soil conditions. Applying dolomite or lime to acidic soils with a pH below 5 is recommended. The hydrolysis reaction of lime ( $\text{CaCO}_3$ ) can produce hydroxyl ions, which can elevate soil pH (Paulose *et al.* 2007). Further, liming has a beneficial effect on soil microbial activity and soil health (Alkorta *et al.* 2003), particularly dehydrogenase activity in acid soil (Lalande *et al.* 2009). The best soil-cum-nutrient management package for mulberry cultivation in acid soil under rainfed conditions is the integration of NPK with FYM, lime based on lime requirement of soil, and foliar spray of boric acid solution (0.1% w/v) (Ray *et al.* 2021) suited to a particular growing environment based on the soil fertility status. Proper nutrient management is the foremost requirement for any crop to maximize the yield (Raising *et al.* 2022).

**Management of saline soil:** While chloride or sulphate predominates in saline soils, carbonate or bicarbonate are the major ions in sodic soils (Vijayan 2009). Saline soil can be physically managed by deep ploughing and leaching the soluble salts with good quality water. In addition, soil and foliar application of potassium have been reported to significantly reduce the toxic effect of salinity as it helps in maintaining both the ions ratio and water balance within the plant and soil, respectively (Martinez and Cerda 1989). An effective screening method for mulberry was proposed by Vijayan *et al.* (2003), wherein, axillary buds were cultured *in vitro* under saline conditions and reported that the *in vitro* screening method is economical and efficient to identify salt tolerant genotypes.

**Management of drought, heat and cold stress:** Mulberry plants exhibit wilting, leaf rolling and premature leaf drop in response to drought stress (Fang and Xiong 2015). Drought also caused reduced seed germination, cell volume and water content, decrease chlorophyll content, premature ageing, and small-dense stomata (Vahdati *et al.* 2009). Premature leaf senescence caused by drought causes serious loss of nutrients in mulberry leaves and loss of their usefulness (Chaitanya *et al.* 2001). Excessive heat leads to wilting and scorching of leaves, thin leaves and reduced photosynthesis, and growth. High temperatures lead to decreased fruit quality and yield in mulberry plants. Exposure to freezing temperatures can damage the plant's tissues, causing wilting, discoloration, and even death (Yadav 2010).

Sowing at a proper time or season is crucial for crop productivity. For instance, timely sown maize crop showed higher yield, nutrient uptake and nutrient use efficiency (Zhiipao *et al.* 2023). Adequate irrigation is critical to prevent wilting and damage to the plant tissues. Mulberry plants

should be irrigated deeply and less frequently to encourage deep root growth, which helps the plants access water from deeper soil layers (Chai *et al.* 2016). While during cold weather, the plants require less water and over-irrigation can lead to waterlogging, and root rot (Kaur *et al.* 2020). Cultural practices such as mulching and shading/netting can also be followed. Judicious application of NPK fertilizers can help improve the plant's tolerance to drought and heat stress (Drobek *et al.* 2019) and cold stress (Rawat *et al.* 2016).

#### *Management of important diseases in silkworm host cultivation*

Numerous diseases target mulberry, which in turn deteriorates the quality of leaves used as feed for silkworms. Most of the diseases cause significant yield losses and a decrease in the leaf's nutritional content, both of which ultimately hinders the production of cocoons and affect silk production, and quality. Resistance breeding in mulberry plants is a promising route, however, screening revealed that there is lack of complete resistance to foliar diseases (Sharma *et al.* 2009). Different management strategies including chemical, cultural, biological are briefly discussed below.

**Physical and cultural methods of mulberry disease management:** Mechanical, thermotherapy and agronomic practices are examples of physical, and cultural approaches to disease prevention. Clean cultivation and removal of plant debris and its destruction by burning to prevent pathogen such as *Alternaria* leaf blight, *Sclerotium* wilt, *Fusarium* leaf blight, etc. greatly reduces the chances of disease incidence. Gupta *et al.* (1999) employed soil solarization for the control of soil-borne pathogens of mulberry in nursery conditions which resulted in inoculum reduction and decline in disease incidence. Intercropping plants like *Tagetes* sp. and *Crotalaria* sp. is another cultural method for effective management of nematodes as roots of such plants form nematicidal substances. Mulberry red rust incidence was found to decrease by interplanting soybean, greengram, ragi and maize between mulberry rows with wider spacing (Govindaiah and Sharma 2001). Soil-borne pathogens causing diseases in the nursery such as collar rot, stem canker and cutting rot demonstrated to be reduced by deep ploughing, and exposing the soil to sunshine after covering it with transparent polythene sheets of 30  $\mu\text{m}$  thickness for roughly 3 months (Sharma and Gupta 2005).

**Chemical methods of mulberry disease management:** Fungicides will continue to be a necessary component of disease management to prevent significant losses in crops. However, due to their high toxicity, fungicides have affected the environment, and the sprayed fungicides are wasted as a result of wind or surface runoff, impacting farmers' economies (Dorjee *et al.* 2023). Carbendazim spray has been found as an effective fungicide against leaf spots and powdery mildew. Dithane M-45, Benomyl and Foltaf at 0.2% as a spray can effectively control diseases like bacterial leaf blight (*Pseudomonas syringae* pv. *Mori*) and bacterial leaf spot (*Xanthomonas campestris* pv. *mori*)

(Sharma and Govindaiah *et al.* 1991). For the control of viral diseases, spraying of insecticides like 0.02% Monocrotophos, 0.1% Dimethoate, 0.1% Dichlorvos or 1% Neem oil azadirachtin are advised to check the vector (Bandyopadhyay *et al.* 2005).

Biological control of mulberry diseases: Biological disease control has been greatly emphasized as a result of increased environmental concern about pesticide use. Culture filtrate of *Trichoderma viride* and *Trichoderma harzianum* significantly led to a reduction of disease intensity (*Phyllactinia corylea*) by 22.84% and 23.42%, respectively (Raja 2010). Sharma and Gupta (2000) reported *Trichoderma harzianum* (Th-1) and *Trichoderma pseudokoningii* (Tp) profoundly reducing the severity of leaf spot and rust by 50 and 44%, respectively. Sridar *et al.* (2000) demonstrated remarkable efficacy of *Bacillus subtilis*, the root rot fungi, *Macrophomina phaseolina*. Soil treatment of *T. harzianum*, *T. viride* and FYM in the ratio of 1:1:50 three times at intervals of 30 days, resulted in the highest degree of plant survival (72.00%), reduction and subsequent spread of the disease (Beevi *et al.* 2010). Strains of bacteria such RC-2 of *Bacillus amyloliquefaciens*, Lu144 of *Bacillus subtilis* were identified as potential antagonists against anthracnose caused by *Colletotrichum dematium* (Xie *et al.* 2020) and *Ralstonia solanacearum* causing bacterial wilt (Ji *et al.* 2008), respectively. Moreover, plant growth-promoting rhizobacteria (PGPR) such as *Azotobacter chroococcum* (Azc-3) and *Pseudomonas fluorescens* (Psf-4) were demonstrated to be efficient against a wide range of pathogens (Gupta and Sharma 2004).

#### *Nanotechnological intervention for mulberry disease management*

Recently nanotechnology has attracted the interest of many due to its myriad benefits and excellent antimicrobial properties. AgNPs against *Cerotelium fici*, *Cercospora moricola*, and *Phyllactinia corylea* showed remarkable inhibitory effects at a concentration of 100 µg/ml (Govindrajua *et al.* 2008). Similarly, Dorjee *et al.* (2022) reported significant efficacy of biogenic copper nanoparticles against Sclerotium wilt at a 100 ppm. AgNPs has been demonstrated to show remarkable antifungal effect against *Colletotrichum* sp., *Pythium aphanidermatum*, *Fusarium oxysporum* and *F. solani* at a low dose (Kim *et al.* 2012) and antibacterial efficacy against a gram-positive and gram-negative bacterial strain (Shirley *et al.* 2010). Recently, Dorjee *et al.* (2023) reported that nanoparticles-based formulation such as Essential oil-grafted Copper nanoparticles have high efficacy against wide host range fungi such as *Fusarium* spp. *Macrophomina phaseolina*, *Rhizoctonia solani* and *Sclerotium rolfsii* at a low dose of 20 mg/litre. Importantly, this high efficacy is achieved without causing severe consequences on the environment, indicating the environmentally-friendly nature of nanotechnological approach at an optimum concentration. The application of nano fungicides in disease control has the potential to curb the losses and also will limit pesticide usage.

#### *Conclusion and future perspectives*

Sericulture is an important agro-based industry for farmer's livelihood in many countries. The quantity and quality of silkworm feeds in the form of leaves obtained from hosts decide the production of quality silk. Leaf harvest is severely affected by various biotic and abiotic stresses. Genetic improvement of the hosts towards stress are essential to enhance the production and productivity. Use of conventional breeding strategies in silkworm hosts face constraints due to heterozygous genetic constitution and long gestation period. On the other hand, tolerance to abiotic stresses is mostly quantitative in nature. The need of stress-resistant cultivars in public domain is huge. Hence, identification of germplasm lines possessing desired trait of interest is the basic step towards development of stress-tolerant cultivars. Creation of genetic variation by means of introduction, mutation, genetic engineering and tissue culture is important in stress breeding. Use of GWAS, genomic selection and QTL mapping strategies will help in identification of superior genotypes besides study of marker-trait association. MAS is an efficient strategy to select desirable lines in parental populations as well in segregating populations. For deeper understanding of the genetics of stress resistance, use of genomic tools such as transcriptomics, proteomics, metabolomics and other advanced OMICS approaches will assist in unraveling the stress resistance/tolerance mechanism. With the stated difficulty in breeding in silkworm hosts, the number of stress-resistance cultivars in the public domain still needs to be increased. Use of resistant cultivars is the cheapest means of management of crops under stress conditions. However, it is inevitable and more sustainable to manage these stresses using inter-disciplinary approach. Here, we review sound agronomic cultural practices for various abiotic stress such as salinity, acidity, drought, heat and cold stress. In addition, we also highlight management practices (physical, cultural, chemical, biological, nanotechnological controls) of diseases especially in mulberry. This review will help the researchers in the field of sericulture and farmers significantly. We proposed the need for a holistic strategy to manage important abiotic and biotic stresses. Suitable agronomic cultural practices and chemical management is required for efficient management of various stress.

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