

Functional Genomics of Abiotic Stresses Tolerance in Flax, *Linum usitatissimum*

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INTRODUCTION

Flax (*Linum usitatissimum* L.; family Linaceae) is an important commercial crop grown across the globe for seed oil (linseed) and bast (stem) fibre (linen). The linseed consists of 35-45% oil and is rich in α -linolenic acid (ALA) and omega-3 fatty acid, soluble dietary fiber, like lignans, and high protein content. Linseed also comprises different minerals and vitamins, especially vitamin E in the form of gamma-tocopherol. Therefore, linseed oil has immense nutritional and therapeutic benefits as feed and food as well as has industrial applications. On the other hand, the flax fibre is chiefly cellulosic material (60-80%) with approximately 5-7% lignin that provides high tensile strength. Fine flax fibres or linen are mainly used in textile industries for cloth, fabrics, bed linen, surgical threads, ropes, twines, and speciality uses *viz.* composites, and automobile industries, etc.

The linseed and fibre type flax are different plant morphotypes cultivated for distinct economic products. These two plant morphotypes vary considerably in plant phenotypes and the growing conditions (Hall et al., 2016). The hot and humid climate is one of the major limiting factors in the climatic adaptability of superior flax cultivars. Similarly, drought and salinity also

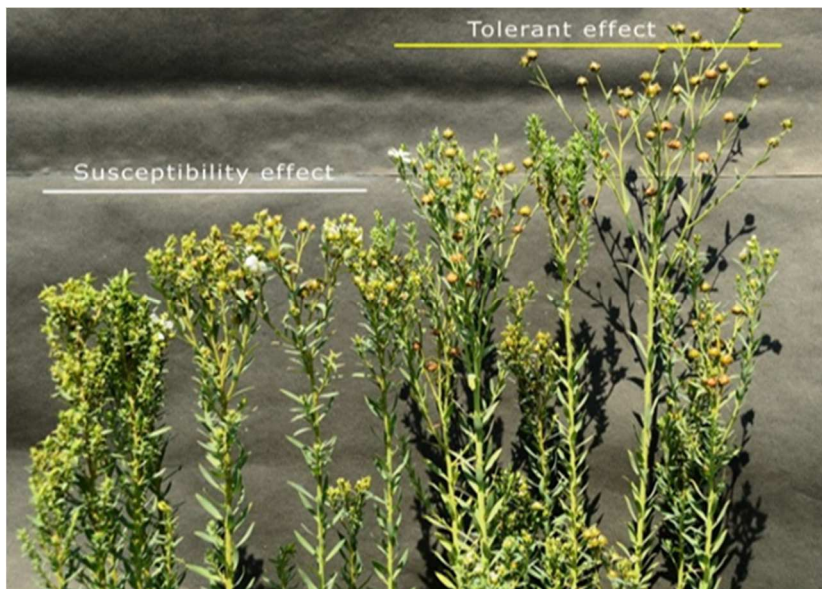


Fig. 1: Effect of heat stress on flax under field conditions

restrict the expansion of global flax cultivation in affected areas. Abiotic stresses in flax, especially at reproductive stages are known to negatively impact oil and fibre yield and quality. A continuous drought or heat stress for 5-7 days during the flowering stage can significantly affect pollen fertility and pod setting (Fig. 1). Although there are inadequate studies, the drought, salinity, or high-temperature stress in flax reported to trigger oxidative damage and elevates membrane lipid peroxidation (Saha et al., 2019a). In general, plants respond to these abiotic stresses through an antioxidant defense system.

Various reactive oxygen species (ROS) scavenging enzymes, such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APOX) are part of this defense machinery. In flax, from the past decade, a significant leap has been achieved in the

availability of various genomic resources, such as genomes, transcriptomes, molecular markers, and linkage maps. Using these resources, the abiotic stress tolerance and wider climatic adaptability in flax through varietal improvement program is foresighted in the near future.

HOW GENOMICS CAN AID TO ACHIEVE STRESS TOLERANCE?

Traditional crop improvement measures have greatly impacted breeding for abiotic stress tolerance in several crop plants. However, those approaches consume considerable time and have been pushed to their limits of any further gain. Applications of advanced genomic technologies, including the next generation sequencing (NGS) - based understanding of molecular mechanisms and gene functions, rapid computational analyses of big sequencing data, artificial intelligence, genome-wide trait associations, targeted gene editing, etc. are revolutionizing the plant breeding and agriculture sciences. A wealth of information is being added and annotated

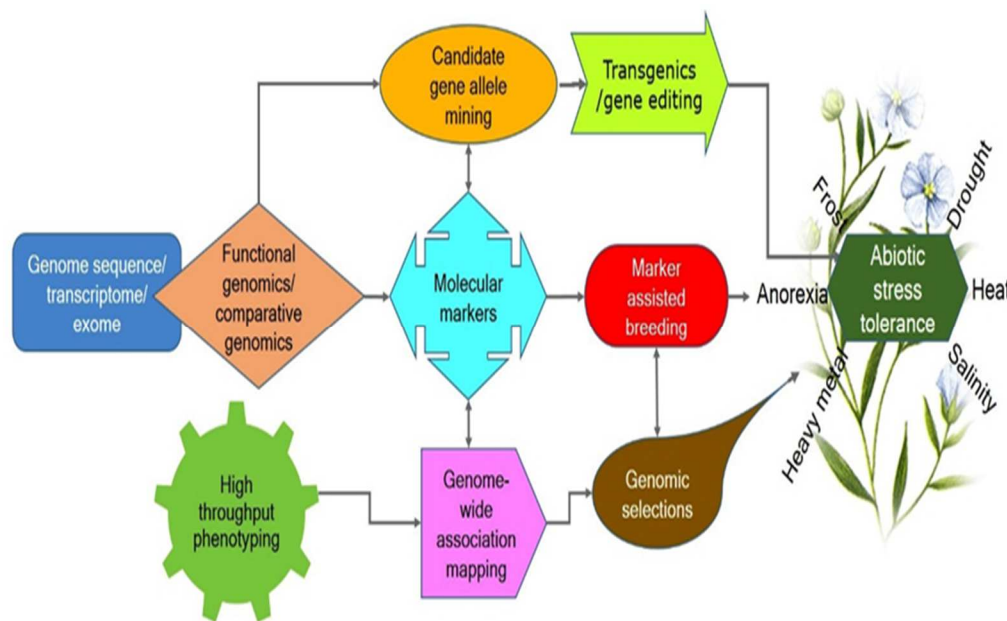


Fig. 2: Genomic-enabled strategies for improving abiotic stress tolerance in flax

rapidly to understand complex biological mechanisms associated with abiotic stress tolerance in plants. In flax, several genomic interventions are reported or are in offing to cater to the needs of improving flax varieties with enhanced tolerance to

various biotic and a biotic stresses (Fig. 2).

THE LEGACY OF FLAX IN RESISTANCE-GENETICS RESEARCH

One of the earliest known significant genetic findings in flax dates back to 1942 when Harold Henry Flor first proposed his famous hypothesis on the ‘gene-for-gene’ relationship. Using *Melampsora lini*, a fungal pathogen of flax rust disease, he went on to propose that ‘for each resistance (R) gene in host there is a corresponding avirulence (*Avr*) gene in the pathogen which confers resistance and vice versa’. Thereafter the genetic and genomics research on flax has traveled a long way. The genome size in flax through flow cytometry was estimated as 373 Mb with haploid chromosome number $n = 15$.

GENETIC AND FUNCTIONAL GENOMIC RESOURCES AVAILABLE IN FLAX

Flax germplasm, both for linseed and fibre types is being maintained in several gene banks, including in India and across the globe (Table 1). Several gene banks comprise more than 46000 flax collections of both linseed and fibre types.

Table 1: Major flax germplasm collection database across the world

Gene banks	No. of accessions
European flax collection at the Czech Republic	11141
The Genetic Resource Information Network of Canada (GRIN-CA)	3378
United States National Plant Germplasm	3004
The Centre for Genetic Resources of the Netherlands (CGN) at Wageningen University	947
Chinese flax germplasm collection	2000
ICAR-NBPGR, India	3000
ICAR-CRIJAF (fibre flax accessions)	400

The majority of the global flax cultivars are for oilseed purposes. Some of the internationally popular oilseed flax cultivars are CDC Bethune, Norlin, Nuline, Linola, etc. Breeding efforts on fibre flax are mostly preferred for the selection of long fibre types, with lodging resistance via stem stiffness and higher fibre content. Some of the global elite fibre flax cultivars are Ariane, Viking, Marina, Evelin, Regina, Belinka, Stormont, Cascade, etc. India's first high-yielding fibre flax variety JRF-2 (Tiara) was developed and released by ICAR-Central Research Institute for Jute and Allied Fibres in 2015.

Using the whole-genome shotgun assembly, the draft genome sequence of the popular Canadian linseed variety CDC Bethune was developed (Wang et al., 2012). A total of 43384 protein-coding genes were annotated from the flax genome. The draft genome was further refined and the contigs were mapped in 15 pseudomolecules or chromosomes (You et al., 2018). Apart from genome sequences, several flax transcriptomes were also developed related to drought stress, osmotic stress, salinity stress, aluminum metal stress, zinc deficiency, and potassium starvation stress. Besides genome and transcriptome sequences in flax, other significant genomic resources include microRNA sequencing, proteome sequencing, genotyping-by-sequencing (GBS), genome resequencing, and Targeted Induced Local Lesion in Genome (TILLING) data. For functional characterization of genes in flax an Ethyl Methane Sulfonate (EMS) mutagenized TILLING population of 4894 mutant lines were generated and phenotyped by the researchers from France. The genome sequence has facilitated the discovery of a large number of molecular markers, like simple sequence repeats (SSRs), single nucleotide polymorphisms (SNPs), etc. A couple of high-density linkage maps were also developed in flax using SSR and SNP markers (Cloutier et al., 2019). At ICAR-CRIJAF, more than 500 regulatory gene-derived SSR markers were developed and demonstrated their utility in differentiating between the Indian and global fibre flax germplasm (Saha et al., 2019b).

CANDIDATE GENES OF ABIOTIC STRESS TOLERANCE IN FLAX

The genome sequence of flax has facilitated the characterization of important candidate gene sequences, linked with fibre and oilseed quality traits. Several candidate genes responsible

for abiotic stress tolerance were also identified and functionally characterized for their role in abiotic stress responses. For example, heat shock factors were identified and demonstrated to confer differential expression patterns under high-temperature stress (Saha et al., 2019a). Similarly, NAC-domain gene families were also characterized to regulate gene expression of multiple abiotic stresses like drought, salinity, cold, and heat (Saha et al., unpublished). A transporter gene family DTX/MATE in flax was identified to mediate abiotic stress response (Ali et al., 2020), while the genome-wide characterization of aquaporin (AQP) genes (Shivaraj et al., 2017) offers the potential to utilize in drought stress. Identification and characterization of many more candidate genes responsible for abiotic stress tolerance are the need of the hour for genetic improvement in flax.

CONCLUSIONS AND PERSPECTIVES ON ABIOTIC STRESSES TOLERANCE IN FLAX

Genetic and genomic resources are being continuously strengthened in flax, which can be efficiently utilized in flax improvement programs. Transgenic plants transformed with superior alleles of genes for abiotic stress tolerance is one of the most common technology for fighting abiotic stresses. The most effective method of genetic transformation in flax is through *Agrobacterium tumefaciens* mediated hypocotyl transformation. Even the use of particle bombardment for transforming hypocotyl tissues successfully produced transgenic flax lines. More recently, a simple method of floral dip transformation using *A. tumefaciens* culture to inoculate unopened flowers of both linseed and fibre flax genotypes was developed with high transformation efficiency. The transgenic technology developed in flax includes herbicide tolerance associated with chemical weed control, fungal disease resistance, insect resistance, and abiotic stress tolerance for local adaptation of climates. For herbicide glyphosate and glufosinate tolerance, transgenic flax lines were engineered for the 5'-enolpyruvylshikimate-3-phosphate synthase (EPSPS) and phosphinothricin acetyltransferase (PAT) genes, respectively. Genome editing is one of the advanced biotechnological strategies that is also being conducted in flax. A combination of CRISPR/Cas9 and single-stranded oligonucleotides (ssODN) was employed by Sauer and his group to engineer EPSPS-coding gene, which confers herbicide, glyphosate-tolerant trait. The genome-editing strategy can be harnessed for abiotic stress tolerance in flax. For example, the HSF genes, which were identified as candidate genes for high-temperature stress in flax, can be edited to impart enhanced adaptation to adverse climatic conditions. Thus, genome editing will be one of the advanced biological approaches for the coming few years to achieve precise and reliable trait development in flax.

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