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A COMPREHENSIVE REVIEW ON OATS' (*AVENA SATIVA* L.) BIOTIC STRESSES AND BREEDING METHODOLOGIES

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SUMMARY

Oats (*Avena sativa L.*), being important *rabi* season and self-pollinating cereal crop, ranked sixth in the world cereal production after wheat, maize, rice, barley and sorghum belonging to the family graminae and hexaploid in nature used worldwide as multipurpose grain, pasture and forage crop. In every aspect, it has an excellent percentage of nutritional components, i.e. starch, rich in proteins and dietary fibres mainly â-glucan content, phytochemicals, trace minerals *i.e.* calcium and iron and vitamins which makes an oat being a superior choice for consumption by humans as well as animals. Moreover, it is highly succulent and palatable crop. The current issue faced by the oats is changing climatic behavior that is resulting into biotic and abiotic stresses. A wide range of desirable QTLs are degenerating due to the negative impact of climate. This review majorly highlight the biotic stresses and breeding strategies in order to inculcate the resilient traits of interest from wild relatives of oats into cultivated ones. Oats breeding lags behind the other major cereals because of its hexaploid nature and modern breeding technologies mainly Marker-Assisted Selection (MAS), Genome Wide Association Studies for stress tolerance (GWAS), Genome Editing Strategies such as CRISPR-Cas 9 have very vast scope in oats breeding and should be used to enhance the oats value and productivity like all other cereal crops.

Key words: Oats, biotic stresses, crop wild relative, molecular markers, CRISPR/Cas9

Oats is an important cool season cereal crop and is self-pollinating in nature. It is the member of genus *Avena* that belongs to the tribe *Avenae* and family *Poaceae* having chromosome numbers of 2n=2x=14, 2n=4x=28 and 2n=6x=42 depending upon its diploid, tetraploid and hexaploid nature with a basic chromosome number of x=7 (Jellen*et al.*, 2006). The genus *Avena* has wide series of wild and cultivated species covering most of its diversity within 25° and 45°N latitude and 20°W longitude and 90°E longitude extending from Canary Islands, through the Mediterranean basin, the Middle East to the Himalayan Mass (Murphy *et al.*, 1992). According to archaeological records, there had been a great association between man and oats and is therefore originated from the admixture of wheat and barley as a weed contaminant and these weedy forerunners are still growing in the winter and temperate climatic regions throughout the world. The wild gene pools and domesticated *Avena* species are the resources for developing the cultivated species: *A. sativa L.*, 2n=6x=42 (the common oat), *A. byzantina* C. Koch, 2x=6x=42 (the red oat), *A. abyssinica Hochst.*, 2n=4x=28 (the Ethiopian oat) and *A. strigosa Schreb.*, 2n= 2x= 14 (the gray oat)(Jellen *et al.*, 2006).

The domestication and cultivation of barley (*Hordeum vulgare* L.) and emmer (*Triticumdicoccum Schubl.*) plays a giant role in woovening the history of origin of oats and was then transported(Murphy *et al.*, 1992). Thehexaploid progenitor of oats *A. sterilisL.* was domesticated in central or northern Europe with its use for forage purpose, later traced back in southwest Asian wheat and barley crops (Zhou *et al.*, 1999).The whole historical evidence of oats is clearlyviewed by Murphy and Hoffman (Murphy*et al.*, 1992).The cultivated hexaploid oat *Avena sativa*, 2n=6x=42 chromosomes, is therefore a natural self-pollinating allopolyploid arose from cycles of interspecific hybridization and polyploidization illustrated in the Fig. 1. It consists of seven pairs of chromosomes (x=7) each comes from three different diploid AA, CC and DD genomes. Firstly, A and C genome diploids combine to form AC genome which is tetraploid in nature that further hybridizes with the

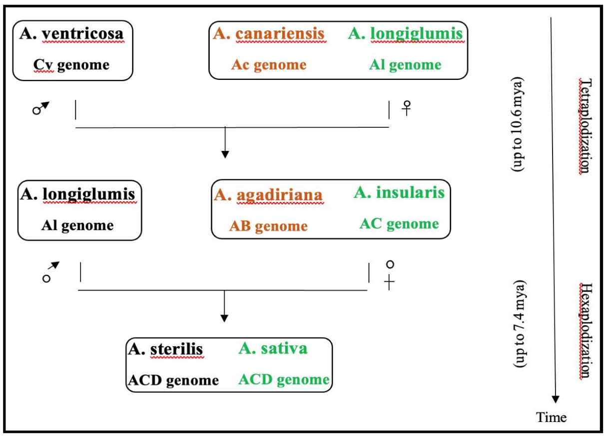

Fig. 1. (mya represents million years ago) This schema chart represents the evolvement of cultivated oat alongwith the maternal and paternal genome diploid and tetraploid contributors based on the organelle (chloroplast and mitochondria) phylogenetic signals.

D genome diploid and hence proposes the hexaploid oat. A phenomenon of chromosome doubling happens at each stage of hybridization in order to stabilize chromosome pairing (Welch *et al.*, 2012).

Recently, a scientific report by Yong-Bi-Futraced the evolution of oats using the multiplexed shotgun procedure performed on chloroplast and mitochondrial genomes of 25 *Avena* species and successfully get maternal lineages. He tries to solve all the unanswered questions such as which diploid and tetraploid species are the genome donors for oat polyploidization and hybridization, what is the pathway and how frequently these polyploidization events do occur? (Fu *et al.*, 2018). The adaptability of the genus *Avena* increases as the ploidy level of the species increases and all the hexaploids are inter-fertile in nature (Murphy*et al.*, 1992). The *Avena*species are broadly categorised into three gene pools, primary, secondary and tertiary, depending upon its fertility of the hybrid formed with the cultivated oat. The primary gene poolcomposed of almost all the hexaploid *Avena*species, the secondary gene poolcomprises of the three tetraploids namely- *A. murphyi, A. maroccana, A. insularis* and the tertiary gene pool bears all the diploid as well as tetraploid species- *A. barbata, A. vaviloviana, A. abyssinica, A. agadiriana, A. macrostachyta.* Oat breeders have highly utilized such species in developing new cultivars as the desirable traits of these species can be well traced (Jellen *et al.*, 2006; Welch *et al.*, 2012).The process of domestication includes plant's germplasm which carries necessary genes helpful in buffering the impacts of changing climatic behaviour and fulfils the need of a breeder by serving as raw material on which whole research depends in creating genetic diversity (Hilli *et al.*, 2023; Kapoor *et al.*, 2020).

Oats stand at sixth position after major cereal crops in world cereal statistics including wheat, maize, rice, barley and sorghum (Hilli *et al.*, 2023; Kapoor *et al.*, 2020) and account for total acreage of9 million hectares with a global production of 19.41 million metric tonnes in which European Union contributes major role by producing about 30% among global production followed by Russia 17%, Canada 14% and minor roles by brazil, Australia,United Kingdom, China (Hilli *et al.*, 2023; https://fas.usda.gov/data/production/commodity/0452000). In India, oats makes its best growth in cool and moist climate but high temperature weather with drought conditions are harmful to the cropmainly from head emerging stage to maturity stage as it reduces the yield by increasing the proportion of empty spikelets. Acidic soil is more suitable for its growth whereas alkaline or water logged soils are harmful (Welch *et al.*, 2012). Nowadays oats is receiving very much attention and its breeding has paved many challenges worldwide. It is highly beneficial crop that is being chiefly used by animals in the form of feed and providesgood hay, silage, pasture, grain feed as well as used by humans as daily diet in the form ofoatmeal and other breakfast meals made from groats. It has multi-cut behaviour and nutritious nature making it suitable for livestock.It has many beneficial health related qualities, a rich source of balance of good quality amino acids, good source of carbohydrates, various dietary fibres such as â-glucan, arabinoxylans and cellulose, lipids, phytochemicals, vitamin E, phenolic compounds and avenanthramides (AVAs). It gives many medicinal benefits to humans as it hashypocholestrolemic and anti-cancerous effect, serves as an immunity booster, provide gluten free diet for celiac diseased patients, relieves constipation in old people, treating UIT infections and so on (Rasane*et al.*, 2015). Oats is also used for industrial purpose in making value added oat based products like cookies, biscuits, infant food, flakes and so on (Rasane *et al.*, 2015; Loskutov *et al.*, 2021; Chawla *et al.*, 2022).

SHIFTS OF CLIMATE CHANGES ON OATS CULTIVATION

Agriculture and changes in climatic behaviour of our atmosphere are interdependent on each other on which our global production and food security is highly dependent. Erratic nature of monsoon, temperature as well as sea level fluctuations, change in the carbon dioxide level, change in the behaviour of flora and fauna are some of the major factors that play a great role in defining the agriculture of a particular region (Raza *et al.*, 2019). Due to variable weather change, year by year, oats possesses a great damage due to high or low temperature and rainfall. The genetic improvement in oats is coping many challenges through acclimatizing new traits. Some researchers had performed a biometric counts of two years 2020 and 2021 then analysed and interpreted the variation that existed in the production of two genotypes namely, SORIN and LOVRIN 1 which are autumn and spring oats respectively (Alina *et al.*, 2021). Climate Smart Agriculture (CSA), the only solution, involves precision farming that is a site-specific management (SSM) which has uplifted the agricultural productivity and adaptivity of crops by developing the climate resilient cultivars through combined effect of plant breeding and biotechnology (Raza *et al.*, 2019; Safdar *et al.*, 2024).

BIOTIC STRESSES AFFECTING OATS PROUDCTION

Stress is an unfavourable condition in a plant due to which it's metabolism, growth and development are adversely affected internally and it's productivity and yield externally. Climate change results into abiotic as well as biotic stress. Biotic stress is the damage caused to a living entity by another living entity such as bacteria, viruses, fungi, parasites, beneficial and harmful insects, weeds as well as cultivated or native plants (Kumar *et al.*, 2021). Due to provision of favourable conditions to biotic stressors, they transform themselves from inactive or non-dominant form to active or dominant form. Therefore, control using resistant cultivars is one of the best method for disease resistance, as inoculum reduction and incorporation of virulence mechanism is the major concern whereas pest incidence is majorly controlled by chemicals as well as IPM technology(Isidro-Sanchez *et al.*, 2020; Valentine *et al.*, 2016).

Oats is infested by various kind of pests which are categorised under foliage feeders, sucking type, soil borne insect pests and nematodes. These are mainly polyphagous and oligophagous rarely monophagous and damage the crop wholly from grain filling stage to harvesting stage (Araya *et al.*, 1992). The important pest is oat aphid which consists of six species-bird cherry-oat aphid *Rhopalosiphum padi*, *Schizaphis graminum*, *Rhopalosiphum maidis*, Russian Wheat Aphid *Diuraphis noxia*, the Rose-Grain Aphid *Metopoliphirun dirhodum* and the Grain Aphid *Sitobion avenae* (Kumar *et al.*, 2017). These aphids damage the oat crop globally in many regions especially USA, Russia, Canada, Australia, Africa alongwith many other pests. Pests include soil insects like wireworms and white grubs are mostly prevalent in USA and Canada, grain eating birds such as *Corvus frugilegus* (rooks), *Columba palumbus* (pigeons), passerine birds and mammals especially rodents like rats and rabbits that mainly graze the crop (Araya *et al.*, 1992; Welch *et al.*, 2012).

A disease is an abnormal condition through which a plant is unable to perform all its functions in a proper manner due to pathogenic attack. It affects the plant wholly from germination to ripening stage by destroying roots, damaging vegetatively growing parts, lowering its growth and ability to reproduce thereby reducing its quality. The favourable optimum conditions for a disease to prevail are well explained by disease triangle- susceptible host, virulent pathogen and favourable environmental conditions, all the three factors prevail at the accurate time period for a disease to occur (Kumar *et al.*, 2021). Among all the devastating pathogens, fungi is the most serious pathogen in case of oat crop(Yumurtaci*et al.*, 2015). Besides fungi, bacteria and viruses also infect the oat crop (Table 1). The most important bacterial diseases are: Halo blight, Stripe blight and Bacterial leaf spot and on the other hand viral diseases include: Oat red leaf, Oat mosaic, Oat blue dwarf and Oat sterile dwarf which are all well explained in the table 1 of this review (Welch *et al.*, 2012). The pathogens started surviving if monogenic nature of resistance is being provided to plants, hence it has become necessary to mine such polygenic traits that may hamper the growth of emerging new races of pathogens and further it may decrease the need of various chemicals and agronomic crop management practices(Isidro- Sanchez *et al.*, 2020).

According to a breeder point of view, three classes of resistances are *Inheritance* of minor (polygenic) and major (monogenic) genes of interest, *Effectiveness* of such genes with respect to gather resistance and *Specificity* of resistance (race-specific verses non-race-specific) (Stuthman *et al.*, 2007). Particular genes of interest termed as *super genes* to be mined from the conserved germplasm resources, wild relatives, gene pools provide suitable results for resistance against many important diseases (Kapoor

TABLE 1

Major biotic stresses of oats production

PESTS OF OATS ALONGWITH THEIR NATURE OF DAMAGE

S. No.	Common name	Scientific name	Family	Order	Host range	Nature of damage
1.	Fruit fly	*Oscinella frit L.*	Chloropi-dae	Diptera	Oligo-phagous	Form 'dead hearts' on young seedlings
2.	Cereal cyst nematode	*Heterodera avenae*	Heteroderi-dae	Tylenchida	Oligo-phagous	Root rots and dies from resulting cyst of dead female
3.	Wireworms	*Agriotes spp.*	Elateridae	Coleoptera	Poly-phagous	Eat young tillers and leaves turn brown and shrivel
4.	Cereal leaf beetle	*Oulema melanopa L.*	Chrysomel-idae	Coleoptera	Oligo-phagous	Feed interveinal tissues on leaves in lanky narrow patterned stripes
5.	Oat aphid	*Rhopalosiphum padi*	Aphididae	Hemiptera	Poly-phagous	Suck phloem tissues and damage inflorescence
6.	Oat thrips	*Baliothrips graminum*	Thripidae	Thysanoptera	Oligo-phagous	Suck juice from grain and feed on leaves, stem and ear causing silver stripes
7.	Leafhoppers	*Carneocephala flaviceps riley*	Cicadelli-dae	Hemiptera	Oligo-phagous	Suck sap from plant cells causing wilting, curling, blotching
8.	Grasshopper	*Melanoplus bilituratus*	Acrididae	Orthoptera	Poly-phagous	Eat by cutting young germinating seedlings
9.	Armyworm	*Chorizagrotis auxilians*	Noctuidae	Lepidoptera	Poly-phagous	Injure oat crop
10.	Cutworm	*Peridroma margaritosa*	Noctuidae	Lepidoptera	Poly-phagous	Injure oat crop

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TABLE 1 Contd
DISEASES OF OATS ALONGWITH THEIR MAJOR SYMPTOM
FUNGAL DISEASES

S. No.	Name of disease	Causal agent	Primary inoculum	Secondary inoculum	Major symptom
1.	Powdery mildew	*Erysiphe graminis avenae*	Through ascospores	Conidia by wind	Greyish white dense mat of cottony mycelium growth on surface of leaves that could be rubbed off
2.	Crown rust	*Puccinia coronataavenae*	Uredospores	Teliospores	Oval to oblong bright orange rust pustules on leaves
3.	Black stem rust	*Puccinia graminisavenae*	Uredospores	Teliospores	Elongated ragged brown pustules mainly on stems and sheaths but smaller on leaves
4.	Leaf blotch	*Pyrenophora avenae*	Long-lived resting mycleium	Conidiophor-es	Occurrence of leaf stripes resulting in reddish-brown necrosis
5.	Speckled blotch	*Leptosphaeria avenaria*	Pycnidia (ascospores)	Macroconid-ia by rain	Leaf blades having purplish spots with orange-brown margins later on enlarge to form blotches
6.	Fusarium blight-scab	*Fusariumgraminaerum*	Macro and micro-condia, chlamydosp-ores	By rain splash or driven by wind	Infecting stem, head gets blightened (appearance of pink mouldy growth), yield reduction
7.	Loose smut	*Ustilago avenae*	Seed-borne	Wind driven	Inflorescence is replaced by mass of smut spores
8.	Anthracnose	*Colletotrichum graminicola*	Through fungus survived in plant debris	Spindle-shaped conidia	Browning of stem, crown tissues, reddish-brown spots on leaves indicating dark sporulatingacervuli
9.	Ergot	*Claviceps purpurea*	Perithecia (ascospores)	Through insects, wind or rain	Grains are changed to purplish-black sclerotial bodies, causes infection in stigma

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TABLE 1 Contd
BACTERIAL DISEASES

S. No.	Name of disease	Causal agent	Primary inoculum	Secondary inoculum	Major symptom
1.	Halo blight	*Pseudomonas coronafaciens*	Gram-negative bacteria	Through bacterial ooze	Seedlings show brown oval spots with dead centre and later on appearance of translucent halo
2.	Stripe blight	*Pseudomonas striifaciens*	Gram-negative bacteria	Through bacterial ooze	Stripes are formed by coalescence of narrow yellowish lesions with halo
3.	Bacterial leaf spot	*Xanthomonas compestris* pv. *Translucens*	Seed-borne	Rain splashes	Water-soaking lesions that coalescence to form brownish streaks later on become translucent

VIRAL DISEASES

S. No.	Name of disease	Causal agent	Primary inoculum	Secondary inoculum	Major symptom
1.	Oat red leaf	Barley yellow dwarf virus (BYDV)	Cultivated and wild grasses	18 species of aphids	Reddish to purple-browncolour appear on leaf tips to downwards, reduced fertile tillering and grain filling, stunting
2.	Oat mosaic	Oat mosaic virus (OMV)	-	Soil borne fungus, *Polymyxa graminis*	Appearance of chlorosis formed from green streaks, cause reduction in tillers, stunting of seedlings
3.	Oat blue dwarf	Oat blue dwarf virus (OBDV)	-	Leafhoppers	Increased tillering, formation of bluish-green colour and enations on leaves, reduction in seed set
4.	Oat sterile dwarf	Oat sterile dwarf virus (OSDV)	-	Plant hoppers (*Javesella pellucida)*	Sterility comes in the oats due to severe stunting, leaf veins gets swelled up and hence formation of enations takes place

et al., 2020). Some examples include, wild species of *Avena*possesses resistance for stem rust against *Puccinia graminis*, stem resistance cultivars are *A. strigosa*, *A. weistii*, *A. barbata*, *A. fatua*. *A.* canariensis, *A. strigosa*, *A. barbata*, *A. vaviloviana*, *A. magna*, *A. murphyi* possesses the resistance against cereal cyst nematode whereas *A. clauda*, *A. pilosa*, *A. ventricosa*, *A. occidentalis* and many more against aphids(Singh *et al.*, 2013).

LEVERAGING CROP WILD RELATIVES (CWR) FOR OATS BREEDING

Cropwild relatives (CWR) are the progenitors that are genetically related to the cultivated crop including wild and weedy ancestral species (Akhalkatsi *et al.*). The basic challenge for making the stress resilient cultivars is the relation of agriculture with the climate change i.e. Genotype×Environment (GE) interaction that deeply affects the crop yield and all the agronomic agricultural practices (Yumurtaci *et al.*, 2015). Crop wild gene pools plays an interesting role in conservation of various important traits that contains the useful alleles for yield and quality improvement as well as stress mitigation and hence serves as a rich source of genetic diversity (Kapoor *et al.*, 2020). Various oats' CWR are conserved in primary gene pool are of hexaploid origin and they serves as a good source of desirable alleles in order to adapt the varying climate. In a study, used joint regression analysis in order to identify some oat wild relatives which they found adapted to changing weather conditions of North-Western Himalayan region of India (Kumar *et al.*, 2022). CWR of *Avena* species are the rich sources of disease resistance and future researchonly resides on digging up of these exotic species. In secondary gene pool, *A. maroccana*h as useful genes for crown rust resistance whereas tertiary gene pool is a good reservoir that contain powdery mildew resistant genes from *A. barbata*, *A. hirtula* and *A. prostrata* species. *A. abyssinica*, *A. barbata*, *A. strigosa*contain crown rust resistance. *A. barbata*contains stem rust resistance. Barley yellow dwarf virus resistance was identified in the species *A. barbata*, *A. macrostachyta* and *A. strigosa* (Jellen *et al.*, 2006). Among the various diploids and tetraploids, *A. strigosa* contain *Pc15, Pc23, Pc92, Pc94* genes and *A. magna* contain *Pc91* gene for crown rust resistance. *Pg6* in *A. strigosa* and *Pg16* in *A. barbata* has been transferred to the cultivated oats against stem rust. Powdery mildew resistance resides in the cultivated species of oats due to *Pm2* gene from *A. hirtula*, *Pm4* from *A. barbata*, *Pm5* from *A. macrostachyta* and *Pm7* from *A. eriantha* species (Mohler *et al.*, 2023). There are some limitations of germplasm which include the insufficiency of full-fledged information about preserved *Avena* species, researchers lose interest in developing new varieties due to lack of basic passport and evaluation data. There is difficulty in maintaining huge accessions as well as regenerating them in the field due to high cost involved. Another important limiting factor is crossing barriers between cultivars in generating and developing new quantitative and qualitative traits that provide hinderance and forbid a breeder to work on the germplasm (Singh *et al.*, 2013; Mohler *et al.*, 2023).

The launch of Victoria lead the way to oats breeding towards disease resistance breeding where crossing of crown rust and smut resistant variety Victoria and stem rust resistant variety Richland was performed by T.R. Stanton (Welch *et al.*, 2012). To cope up with the limitations described earlier and when intraspecific combinations are not feasible, different germplasms when crossed with the cultivated species produces a wide range of genetic diversification among species consisting of desirable variety of alien genes of required characters and has been proved a victorious step towards crop enhancement (Singh *et al.*, 2013). A large amount of pioneering works were done in transferring the disease resistance from various wild species to cultivated ones, some examples include transferring of crown rust resistance from the varieties *A. sterilis* and *A. strigosa*, stem rust resistance were being transferred from *A. sterilis*, *A. barbata*, *A. strigosa* and *A. longiglumis*. *A. sterilis* and *A. barbata* also includes resistance to powdery mildew alongwith the varieties *A. hirtula*, *A. prostrata*, *A. ventricosa* (Singh *et al.*, 2013).

Traditional methods of producing new cultivars were time consuming and reduces the possibility of evolving new varieties. But now breeders are trying to use certain techniques like double haploidy (DH) to develop climate resilient cultivars with enhanced biotic stress resistance in many crops such as barley, wheat, maize, rice, potato, onion. Oats still remain defiant of all these methodologies due to some reasons that include the presence of pre- and post-zygotic barriers. Double haploidy technique is highly advantageous in cereal crops in developing climate resilient varieties by rapid generation of homozygous lines which accelerates the breeding process and reduces time. With the help of double haploidy technique, genetic maps can be formed and moreover it would be a quick step in identifying the desired QTLs, when combined with molecular markers would be greatly feasible in developing new biotic and abiotic stress tolerant varieties. It enables the quick selection of transferrable QTLs and stabilize those traits mainly of fungal diseases and pests resistance. DH lines is making its huge importance in plant breeding as it can be used in conjunction with molecular-assisted selection and genomic selection in generating genetic diversity by introducing novel traits as well as with transgenic approaches or gene editing using CRISPR-Cas9 further in enhancing the ability of agriculture to adapt the going on climate crisis. A number of attempts in using such technology in oats are in practice among which latest attempt has been on the two varieties: *A. longiglumis* and *A. insularis* that were sequenced from first QTL linkage map of oat (Warchol *et al.*, 2023; Marcinska *et al.*, 2013).

ASSIMILATION OF GENOMIC APPROACH FOR CROP RESILIENCE

Molecular Marker-Assisted Breeding

Oats being a self-pollinating crop requires pure homozygous lines which took eight generations for a resulting progeny to be genetically similar according to conventional plant breeding approaches which were earlier based on hybridization and the benefits of increased yield and productivity, enhanced quality and biotic and abiotic resistance in the cultivars were being attained by a long track into required progeny after repeatedly evaluating each generation for selection based on their phenotypes (Isidro-Sanchez *et al.*, 2020).There was a change from traditional breeding to molecular breeding where quantitative trait loci (QTL) are being enlightened for their identification and then selection by using molecular markers that has been made it possible to sort out those genes from the chromosomes which are giving additional effects in the quantitative traits, a major requirement for making these molecular techniques successful (Singh *et al.*, 2013). QTL mapping is done on the populations resulting from the backcrossing or double haploidy techniques (Stuthman*et al.*, 2007) and it is not just the discovery of polymorphic, cheap, user friendly and high outturn markers but it is more important for a marker to be associated with that desirable phenotypic QTL i.e. trait specific molecular markerfor its efficiency and easiness (Isidro-Sanchez *et al.*, 2020).

Markers are very effective in disease resistance breeding as it saves the time in struggling for a number of inoculations to be made artificially for a disease (Valentine *et al.*, 2016). First molecular marker which has helped many oat researchers in their work was RFLP discovered by Botstein et. al. in 1980 that has paved a way to discover crown rust and stem rust resistant genes (Kapoor *et al.*, 2020). The project supervised under Catherine Howarth and Tim Langdon was started with 110 simple sequence repeats (SSR) markers then became useful in oats. Later on, 40 SSRs too showed polymorphism nature in oats which were originally of barley, wheat and ryegrass markers. This project in collaboration with Svalof-Weibull in Sweden and a genomics company of Quebec developed a total of 250 markers which were monomorphic as well as polymorphic (Valentine *et al.*, 2016). Different scientists have dug out several resistant genes for major diseases, examples include for crown rust resistance genes mapped against the pathogen *Puccinia coronataf. sp. avenae*were *Pc38, Pc39, Pc48, Pc54, Pc59, Pc58, Pc68, Pc71, Pc91, Pc92, Pc94, Pcq2*. Powdery mildew resistant genes against *Blumeriaagraminisf.sp. avenae* were identified on various chromosomes like *Pm1*- chromosome *1D*, *Pm3- 17A*, *Pm6- 10D*, *Pm7-13A*, *Pm8- 4C* and many more explained by Isidro-Sánchez *et al.,* 2020 alongwith the genes mapped for oat stem rust and barley yellow dwarf virus (Isidro-Sanchez *et al.*, 2020).

Oats QTLs were identified and mapped for Pyrenophora leaf spot resistance. Linkage map for a total of 96 populations was constructed on which 24 SSR markers were associated with the help of QTL cartographer, hence these results get the focus of upcoming oat researchers on those genomic regions that are responsible for disease resistance(Hilli *et al.*, 2024). A total of 31 SSR markers out of 170 used in 96 oat populations for dual purpose breeding (10 traits of fodder and 8 of grain purpose) among which 100 alleles were identified (Kaur *et al.*, 2024).Another great contribution made recently using SNP markers by linking them with Pl 258731 accession of *A. strigosa* then mining and mapping of all the QTLs of genomic region that are responsible for the resistance against crown rust disease further introgressing the resistance into desired germplasm *A. sativa* (Chowdhury *et al.*, 2024).

Marker-Assisted Selection (MAS), Marker-Assisted Backcrossing (MABC), Marker-Assisted Recurrent Selection (MARS) and Marker-Assisted Gene Pyramiding are all parts of Marker-Assisted Breeding (MAB) (Kapoor *et al.*, 2020). As discussed earlier about artificial inoculations of a particular disease during development of a disease resistant cultivar and thereby in order to set up the disease resistant gene to get appeared in that particular germplasm, MABC proved a successful journey behind the acceleration of biotic stress tolerant breeding(Isidro- Sanchez *et al.*, 2020). Following are the various steps involve in this technique-formation of F_1individual from the cross of recurrent and donor parent, for selection purpose performing back-cross of $F_1$with the recurrent parent and then selfing the progeny obtained in order to generate nearly homozygous lines so that the effect of our gene of interest can be visible. This is the successful method to obtain the near-isogenic lines (NILs) or chromosomal segment substitution lines (CSSLs)(Chandra *et al.*, 2024).Another important kind of Marker-Assisted Selection (MAS) is Genomic Selection (GS), a powerful tool used for efficiently accelerating stress resilience breeding in cereals (in case of oats, yield and quality of dual-purpose crop, disease resistance and for adapting the changing climate) of complex traits mainlythat has enhanced the genomic gain(Mallikarjuna *et al.*, 2022). The Marker-Assisted Selection (MAS) was limited only to the major QTLs responsible for the breeding purpose and do not take into consideration the minor ones that have also some additional effect for extensive phenotypic evaluation purpose which then rther enhanced with the introduction of genomic selection (GS) that enabled to study all the small effect QTLs in a genome and improve the selection process (Mallikarjuna *et al.*, 2022; Hilli *et al.*, 2024). Asoro *et al.* in 2011 published his work on five traits of 446 elite North-American oat lines using 1005 Diversity Array Technology (DArT) markers and two Genomic Selection methods namely, RR-BLUP (Ridge Regression-Best Linear Unbiased Prediction) and BayesCð (Isidro- Sanchez *et al.*, 2020; Asoro *et al.*, 2011). Sørensen *et al.* optimizes genetic and phenotypic variety as well as genetic similarity between training and testing populations of Norwegian 1124 oat lines (Sorensen *et al.*, 2023).

Mapping of genomic regions for stress tolerance: Genome-Wide Association Studies (GWAS)

The main objective of Genome-Wide Association Mapping (GWAS) is to study the complex traits accounting for mainly disease resistance by associating all the genotypes responsible in order to spot the phenotypes(Isidro- Sanchez *et al.*, 2020).It is essential for a phenotypic Quantitative Trait Locus (QTL) to be in connection with the marker locus and thereby in order to analyse the result ofeffect caused by the QTL by taking into consideration many hundreds and thousands of polymorphisms.It has a very high resolution which enables to detect the phenotypic variation or character responsible by the Single Nucleotide Polymorphisms (SNPs) inside genes (Mallikarjuna *et al.*, 2022; Chandra *et al.*, 2024). Generally, the major factors of success to GWAS are enhanced statistical techniques, decreased probability of the occurrence of Type-I error and the interpretation of the association between population structure and cryptic relatedness, which means individuals are more closely related to the population of unrelated individuals that act as a confounding factor in the case of Genome-Wide Association Studies. With the availability of highly polymorphic molecular DNA markers and statistical tools, GWAS has created its place and interest in oat breeding. Most of the GWAS studies of oat breeding are focused on quality enhancement and disease resistance traits by relating the genotype of such complex traits to detect its resulting phenotype and as a result diseases are identified due to existence of variation among various genotypes (Isidro- Sanchez *et al.*, 2020). GWAS was firstly used by Newell et. al. working on the â-glucan content of 431 oat genotypes and found three DArT markers linked to the trait. Esvelt Klos et. al. 2017 worked crown rust resistance using 631 sample size and found 29 SNPs on new linkage groups which were not previously identified by any of the researcher (Isidro-Sanchez *et al.*, 2020).

GWAS has created strong marker-trait bonds with the help of various statistical techniques and configurations of desired targeted population. It is highly helpful to gain knowledge about many complex traits of crops at genome level. Using the traditional GWAS approach, many new methods were discovered using such association principles between the desired traits and DNA markers. These are Nested Association Mapping (NAM), Multi-parent Advanced Generation Intercross (MAGIC), Genomic Control (GC) and Structured Association (SA). Newly, a recent approach called Multi-Parent Populations (MPPs) have paved its way that combine genetic diversity with an ease to control population structure and has been used with a great success in various cereal crops for breeding and genetics purpose (Chandra *et al.*, 2024).Genome-Wide Association Studies (GWAS) have been increasingly employed to investigate important traits in oats, facilitating the identification of genetic markers associated with various agronomic and disease resistance characteristics. For instance, Canales et al. (2021) conducted a GWAS on 669 landraces and 40 cultivated varieties of oats using SNP markers to study heading date, a key trait influencing growth cycle and adaptation. Similarly, Yan *et al.*, (2023) performed a GWAS on 319 oat accessions to identify SNP markers associated with grain weight and size, crucial traits for improving yield and quality in common oat. GWAS has also been applied to morphological traits such as hullessness and lemma color in oats, as demonstrated by Wang *et al.* (2023) in a study involving 288 lines. Additionally, Zhou *et al.* (2024) used SNP markers to examine coleoptile length, an important factor in seedling establishment, in a sample of 243 oat accessions. Disease resistance has also been a focus, with Schurack*et al.* (2024) utilizing DArt markers to identify a powdery mildew resistance gene (Pm13) on chromosome 1D in 250 oat lines. Finally, Sørensen *et al.* (2024) applied SNP-based GWAS in a study of 541 oat lines to uncover genetic loci associated with Fusariumgraminearum resistance, a major pathogen affecting oat crops. These studies underscore the power of GWAS in oat breeding, providing valuable insights into genetic loci that can enhance both agronomic traits and disease resistance in oats.

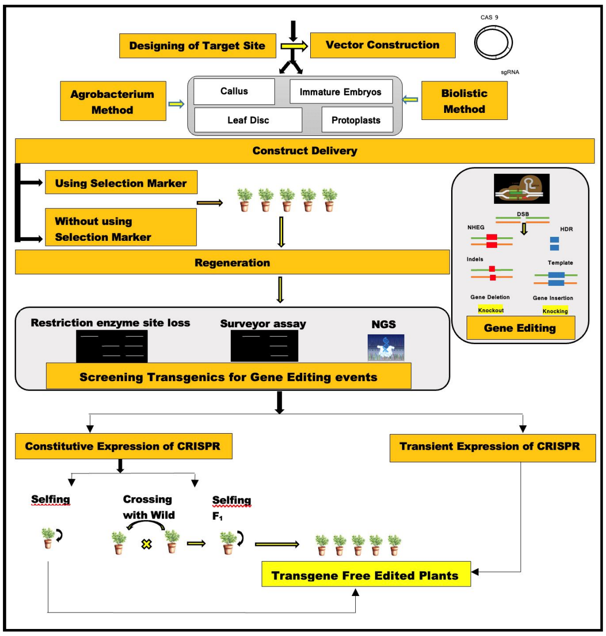

Fig. 2. Detailed flowchart of CRISPR/Cas9 mediated genome editing in plants.

Potential of CRISPR/Cas9 for oats improvement

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) is most widely used tool for genome editing nowadays, shown in figure 2 of this review. (Mallikarjuna *et al.*, 2022; Wang *et al.*, 2023; Jiang *et al.*, 2017). CRISPR-Cas9 technology creates small indels(insertions, deletions or point mutations) and hence being used in the multiplexed editing of crop species in mainly two cases: crop plants where multiple copies of target genome are present (in wheat and oats) and the domesticated crop plants where multiple targeting of desired genome is required (Wang *et al.*, 2023; Jiang *et al.*, 2017).This technology is making a number of groundbreaking advancements in various categories of crops such as cereals(majorly rice, wheat, maize) (Maharajan *et al.*, 2022; Basu *et al.*, 2023), oil crops(Arabidopsis, Camelina, Peanut, Rapeseed, Soybean, etc) (Li *et al.*, 2024) andin horticultural crops (tomato, banana, potato) (Tripathi *et al.*, 2024; Ortega- Salazar *et al.*, 2024; Prasad *et al.*, 2024; Sood *et al.*, 2024). Regarding oat research, an improvement in gene sequencing has solveda major issue and an important breakthrough required for oat breeders was hidden oat genome. The results had shown that the hexaploid *Avena sativa* (AACCDD) is over 11 Gb genome size comprised of diploid *Avena longiglumis* (AA) and tetraploid *Avena insularis* (CCDD) with their genome size 3.7 Gb and 7.3 Gb respectively (Hilli *et al.*, 2023). This has opened many doors for CRISPR technology to enter into oats improvement (Li *et al.*, 2022; Maharajan *et al.*, 2022).

Classical breeding methods like cross breeding, mutagenesis breeding and so on cannot fulfil the requirements for long term benefit of resistance breeding. The promisingsolution to such problem is inculcating bioinformatics into plant breeding by using genome editing technologies (CRISPR/Cas) with precise genome modifications and further reducing the breeding life which has become the major objective in current research for resistance breeding due to great demand of new stressors adaptive cultivars(Li*et al.*, 2022; Chandra *et al.*, 2024). Recently, Thomson Donoso successfully edited *Avena sativa* using CRISPR-Cas9 system for beta-(1,3;1,4) glucan component. The inverse relationship demonstrated by Singh *et al.* 2017 in Thaumatin-Like protein 8 (TLP8) and barley's beta-glucan content build up an urge to conduct an investigation for the same relationship in *Avena sativa*. His two major research objectives were: firstly, determination of correlation of beta-glucan content with the common oats by finding out the presence of TLP8 ortholog genes and secondly, discovering the association of Thaumatin-like protein with the beta-glucan content of oats variety Park by knocking out ortholog gene TLP8 with CRISPR-Cas9 tool for genome editing (Donoso2021). With this victorious move towards precision oats breeding, researchers are being inspired and they are developing their keen interest for using CRISPR-Cas9 genome editing technology for quality and resistance breeding. As by growing research, oats genomehas become a reality which is simultaneously creating an abundant scope for site-specific mutagenesis approach precisely using CRISPR-Cas9 (Saini *et al.*, 2019).

Oats for future

Occurrence of genetic uniformity in agricultural crops due to drastic reduction in highly profitable agronomical traits as well as practicing monoculture of major cereal crops (rice-wheat system)are the main reasons that are responsible for the loss of our agricultural biodiversity (Yumurtaci *et al.*, 2015). On an account of great demand for high yielding, quality enhancing and stressor resistant oat cultivars due to an intense alteration in G × E (Genotype Environment) interaction had given a new vision to oat breeders in widening the oats research(Kapoor *et al.*, 2020). Advancements in plant breeding give rise to the new theories in totipotency and led to the discovery of naturally occurring haploids that later on resulted in haploid and double-haploid (DH) plants. All such advanced technologies like double-haploidy, speed breeding, mutation breeding in combination with new genetics and bioinformatics strategies like genome editing using CRISPR-Cas9 have proved a robust approach in cereal crops. This publication has driven all the interest of oat scientists towards itself showing a new golden opportunity for accelerating and expanding the oats research by adopting all such sturdy and vigorous tools with promising smooth future research.

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