Journal of Genetic Engineering and Biotechnology 22 (2024) 100376

Contents lists available at ScienceDirect

Journal of Genetic Engineering and Biotechnology

journal homepage: www.elsevier.com/locate/jgeb

Whole genome resequencing unveils low-temperature stress tolerance specific genomic variations in jute (Corchorus sp.)

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ARTICLE INFO

Keywords:
Corchorus. olitorius accession no. 2015 (acc. 2015)
Corchorus. olitorius var. O9897
Single Nucleotide Polymorphisms (SNPs)
Insertion Deletions (InDels)
Copy Number Variations (CNVs)
Gene Ontology (GO)

ABSTRACT

Jute (*Corchorus* sp.), a commercially important and eco-friendly crop, is widely cultivated in Bangladesh, India, and China. Some varieties of this tropical plant such as the *Corchorus. olitorius* variety accession no. 2015 (acc. 2015) has been found to be low-temperature tolerant. The current study was designed to explore the genome-wide variations present in the tolerant plant acc. 2015 in comparison to the sensitive farmer popular variety *Corchorus. olitorius* var. O9897 using the whole genome resequencing technique. Among different variations, intergenic Single Nucleotide Polymorphism (SNPs) and Insertion-Deletion (InDels) were found in the highest percentage whereas approximately 3% SNPs and 2% InDels were found in exonic regions in both plants. Gene enrichment analysis indicated the presence of acc. 2015 specific SNPs in the genes encoding peroxidase, ER lumen protein retaining receptor, and hexosyltransferase involved in stress response (GO:0006950) which were not present in sensitive variety O9897. Besides, distinctive copy number variation regions (CNVRs) comprising 120 gene loci were found in acc. 2015 with a gain of function from multiple copy numbers but absent in O9897. Gene ontology analysis revealed these gene loci to possess different receptors like kinases, helicases, phosphatases, transcription factors especially Myb transcription factors, regulatory proteins containing different binding domains, annexin, laccase, acyl carrier protein, potassium transporter, and vesicular transporter proteins that are responsible for low temperature induced adaptation pathways in plants. This work of identifying genomic variations linked to cold stress tolerance traits will help to develop successful markers that will pave the way to develop genetically modified cold-resistant jute lines for year-round cultivation to meet the demand for a sustainable fiber crop economy.

1. Introduction

Jute is an annual, dicotyledonous, fiber-yielding plant of the genus *Corchorus*, belonging to the Malvaceae family.[1] Jute called the "Golden Fibre of Bangladesh", is one of the main export-earners for Bangladesh, as the country remains the world's second-largest producer of raw jute and fiber.[2] It is the cheapest and second most important natural source of bio-based fiber after cotton, in terms of production, usage, and availability. Among 170 species of jute,[3] *Corchorus olitorius* (tossa or dark jute) and *Corchorus capsularis* (white jute) are the most dominant ones.[4] Perfect jute fiber yield is obtained when the jute plants are grown at temperatures between 24° −37° C.[5] The growth declines with a decrease in temperature because biological responses in jute are temperature-dependent.[5] As a self-pollinated plant, the natural genetic variability of jute is narrow[6] but some varieties of this tropical plant have been found to be low temperature tolerant (12°–16°C).[6–7] Therefore, identifying low-temperature tolerant varieties and exploring their underlying genetic basis is necessary to ensure the demand for jute and its profitable cultivation throughout the year. A previous study on DNA fingerprinting, using different primer combinations of

Abbreviations: ***BAM***, Binary alignment map; ***BWA***, Burrows-Wheeler aligner; ***CNVRs***, Copy number variation regions; ***CTAB***, Cetyltrimethylammonium bromide; ***Gb***, Gigabase; ***gDNA***, genomic De-oxyribonucleic acid; ***GFF***, General feature format; ***GO***, Gene ontology; ***InDels***, Insertions-deletions; ***KAAS***, KEGG Automatic Annotation Server; ***KEGG***, Kyoto Encyclopedia of Genes and Genomes; ***NGS***, Next-Generation Sequencing; ***SNPs***, Single-nucleotide polymorphisms; ***Ts***, Transitions; ***Tv***, Transversions; ***VCF***, Variant call format.

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https://doi.org/10.1016/j.jgeb.2024.100376
Received 28 November 2023; Received in revised form 23 March 2024; Accepted 27 March 2024

randomly amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) produced 93 polymorphic fragments with distinguished banding patterns in the two low-temperature sensitive varieties (O9897 and O4) and four tolerant jute accessions of *C. olitorius* (Acc. No.1540, 1805, 1852 and 2015).[7–8] The employment of techniques like next-generation sequencing (NGS) is essential for its improvement and for determining differences between cultivars/accessions at the molecular level.[9] Such sequencing data can unravel not only the existing molecular basis of regulatory pathways[10] but also the novel adaptation mechanisms that are involved in plant stress responses[11]. Plants adopt different functional traits by natural selection[12] and in response to environmental stimulants[11] linked with genetic markers present in the genome.[13]

When a reference genome is available for a species, sequencing of other individuals or varieties of the same species termed whole-genome resequencing[14] allows for the discovery of various DNA markers such as SNPs, InDels, and copy number variations (CNVs).[15–16] Although jute research faces difficulties due to its structural and genetic complexities, the availability of jute genome sequences[17–20] has enabled a variety of genomic studies in recent years. By performing *de novo* assembly of the chloroplast genome of *C. capsularis* and *C. olitorius*, Fang et al. (2021) identified 2,417 SNPs and 294 InDels.[21] InDel markers were developed by Zhang et al. (2017) from cellulose content-related expressed sequence tags (ESTs).[22] An insertion-deletion (InDel) polymorphism database was also developed for jute by Yang et al. (2018) through transcriptome analysis.[23] However, to the best of our knowledge, there is no report on a comprehensive genomic variation profile for low-temperature sensitive and tolerant species of *C. olitorius*. Temperature changes can cause variations in numerous physiological, biochemical, molecular, and metabolic processes during cold acclimation to adjust their metabolism to address the low-temperature stress.[24] Hence, understanding low-temperature adaptation is crucial to developing jute plants capable of growth in the mild winters of the tropical regions where it is cultivated. In this study, we tried to utilize the whole genome resequencing data of the two varieties of *C. olitorius* to find distinct genetic differences between the low-temperature sensitive variety O9897 and low-temperature tolerant variety acc. 2015 to determine the genetic basis (in terms of SNPs, InDels and CNVs) of low-temperature tolerance in jute. Hence, this study of the identification of naturally occurring genetic variations will assist in generating important cold-tolerant trait-specific markers that will help plant breeding and genetic engineering to develop genetically modified plants with adaptive features.

2. Methods

2.1. Plant materials

For the whole genome sequencing, seeds of two jute *C. olitorius* varieties O9897 and acc. 2015 were collected from the seed bank of the Molecular Biology Lab at the Department of Biochemistry and Molecular Biology, University of Dhaka. Seeds were allowed to germinate in petri dishes containing moist tissue paper as the support for 3 days at room temperature. During this initial germination period, the seeds were watered sufficiently. Following 3 days after seed germination, seedlings were collected for DNA extraction.

2.2. Genomic DNA extraction

Genomic DNA (gDNA) of O9897 and acc. 2015 were extracted according to the cetyltrimethylammonium bromide (CTAB) method,[25] with slight modification involving the use of 0.3 % (v/v) β-mercaptoethanol instead of polyvinylpolypyrrolidone and 0.2 % (v/v) β-mercaptoethanol. The quantity and quality of each extracted DNA sample were evaluated by measuring double-stranded DNA concentration and the 260/280 and 260/230 nm ratios, respectively, using NanoDrop One spectrophotometer (NanoDrop Technologies, Wilmington, Delaware, USA). The integrity of the gDNA samples was assessed by 1 % agarose gel electrophoresis followed by fluorescent tagging (ethidium bromide) of gDNA.

2.3. Whole genome sequencing, library preparation, and NCBI accession

DNA libraries were prepared and sequenced (MACROGEN CO., LTD., Geumcheon-gu, Seoul, South Korea). Approximately 350 bp inserts were constructed using Illumina® TruSeq® Nano DNA Library Prep kits (Illumina, San Diego, CA), and 151-bp paired-end reads were generated using an Illumina NovaSeq 6000 sequencing platform (Illumina, San Diego, CA). Final processed genome data from the two varieties were submitted to NCBI SRA (sequence read archive) database under BioProject ID: PRJNA951086.

2.4. QC, mapping, and post-mapping processing

FastQC (version 0.11.9)[26] was used to perform some simple quality control checks to verify per base sequence quality, per sequence quality scores, GC content, sequence length distribution, etc. After verifying read qualities using FastQC, Trimmomatic version 0.39[27] was used to perform adapter trimming, quality filtering, and Poly-G removal from both ends of raw sequences with a Phred score greater than 20 (Phred-like score). The obtained clean reads were mapped to the jute genome (GenBank Accession: GCA_001974825.1)[17] as a reference using Burrows-Wheeler aligner (version 0.7.17, BWA-MEM algorithm) with default parameters[28]. Various tools of the SAMtools (v1.15)[29] and Picard (v2.18.7) package were utilized sequentially for post-processing (e.g., coordinate-based sorting, correction of read pairing information, duplicate read removal) of the mapped reads.

2.5. Variant calling, annotation, and functional enrichment analysis

Short variants, including single nucleotide polymorphisms (SNPs) and small insertions-deletions (InDels) calling, were detected using the Bayesian-based FreeBayes software (v1.3.6)[30] with a minimum base quality of 20, and minimum mapping quality of 20. Quality-based filtering was applied to the resulting variants set using VCFtools[31] to generate a high-confidence final set of variants for downstream analysis.

The effects of the final short variant list were annotated based on their genomic position using the SnpEff software (v5.1).[32] Since there is no database for *Corchorus olitorius* among the pre-built databases for SnpEff, a specific database was prepared using the reference genome and its GFF file (version 3)[17] according to the database building guidelines (https://snpeff.sourceforge.net/SnpEff_manual.html#databases) in SnpEff. Then, SnpEff annotated the genetic variants and predicted their effects on genes and proteins.

The list of genes related to each variety's unique variants was subjected to gene ontology (GO) enrichment analysis using the database for Annotation, Visualization, and Integrated Discovery (DAVID) version 7.0.[33] The calculated p-values were corrected using the Benjamini correction for multiple testing and the most enriched terms were obtained.

2.6. Assessment of copy number variations

Copy number variations in the genome of the two jute varieties were identified from the paired whole-genome sequence data using two tools named DELLY2[34] and Control-FREEC software v11.6.[35] Aligned reads of O9897 variety (low-temperature sensitive) were used as control and acc. 2015 (low-temperature tolerant) variety was used as the sample in Control-FREEC software having a mapping quality of at least 30 with the following parameters: break point thresh-

old = 0.8, coefficient of variation = 0.05, break point type = 2, ploidy = 2, sample purity = 1. Both DELLY and Control-FREEC were used to identify CNV positions and were categorized into different pathways using KAAS – KEGG Automatic Annotation Server.[36]

3. Results and discussion

3.1. Whole genome sequencing and mapping of O9897 and acc. 2015

Whole-genome resequencing of two varieties produced a total of 14.95 Gb and 14.21 Gb data for O9897 and acc. 2015 respectively with 30X sequence depth (NCBI SRA database under BioProject accession ID: PRJNA951086). Fig. 1 represents the information of whole genome resequencing data of *C. olitorius* low temperature sensitive (O9897) and tolerant (acc. 2015) jute plants.

After trimming, 99.09 % of the raw reads, with an average length of 140 bp, were kept and mapped the jute reference genome "*Corchorus olitorius* var O4".[17] Approximately 86.11 % and 79.01 % of clean reads were mapped for *C. olitorius* O9897 and acc. 2015 (Table 1), respectively, to the reference jute genome (GenBank Accession: GCA_001974825.1)[14] which is significant according to the requirement (range between 70 % and 90 %) of variant calling.[37] The processed and mapped reads were then subjected to variant detection following the variant calling pipeline[37] summarized in Table 1.

3.2. Identification of SNPs and InDels in O9897 and acc. 2015

High-quality SNP and InDels were identified where the frequency of SNPs was approximately one per 302 bp and 325 bp for O9897 and acc. 2015, respectively, while the frequency of InDels was approximately one per 586 bp for O9897 and 619 bp for acc. 2015 (Table 1). The result implied a more frequent density of SNPs than the density of InDels which is supported by earlier reports.[38–39]

Among the total SNPs, the ratio of homozygous and heterozygous variants was 1:5.97 (919873 versus 154,000 SNPs) for O9897 and 1: 4.30 (803527 versus 186,886 SNPs) for acc. 2015. The ratio was also analyzed within the identified InDels which resulted in a homozygous versus heterozygous ratio of 1:1.63 (194577 versus 319104) for O9897 and 1:1.42 (202377 versus 287371) for acc. 2015 (Table 1). These results reflect the fact that the ratio of heterozygous to homozygous (het/hom) can be higher in detected SNPs than in InDels[40] and heterozygous variations can surpass homozygous variations[41]. In addition, higher heterozygous mutations were observed in previous studies of deleterious mutation in rice, soybean, tomato[42], and whole genome resequencing of chickpea parental line.[43] In further analysis, the overall quality of identified SNPs was found to be significant in terms of the transition/transversion (Ts/Tv) ratio which was 1.91 for O9897 and 1.87 for acc. 2015 of *C. olitorius*. A greater than 0.5 ratio is justified as it is known to differ from species to species and even among individuals of the same species.[44] The number of SNPs with transitions (A/G and C/T) was higher than SNPs with transversions (A/T, A/C, G/T, and G/C) (Table 1). The observed transition bias over transversion is the most common type of mutation and was evident in previous studies of wheat, maize, lotus, etc..[45] In both our samples, a higher occurrence of C to T transition was observed (Table 1). This is because 5-methylcytosine deamination results in thymine formation, which is significantly more difficult to be detected by repair enzymes.[46]

3.3. Functional annotation of identified SNPs and InDels

Functional annotation of the identified variants was carried out using the variant effect prediction software SnpEff[32] and they were classified into several functional categories (e.g., stop_gained, start_lost, high, low, missense, nonsense, etc.) (Fig. 2**A**) and locational (e.g., genic regions: exons, introns, splice sites and intergenic regions: stretches of DNA that lie between genes) (Fig. 2**B**). Of the total SNPs and InDels found across the two varieties, most SNPs (~45 %) and InDels (~43 %) were located within the intergenic regions, and the rest were in the genic regions of the genome (Fig. 2**C**). The number of SNPs harboring exonic regions (3.59 % for O9897 and 3.56 % for acc. 2015) (Fig. 2**C**) was significantly higher than the number of InDels in those regions (1.95 % for O9897 and 2.02 % for acc. 2015) (Fig. 2**C**).

Based on their impact on respective regions in the genome[47], the SNPs and InDels were characterized into four types: high impact (affecting splice sites, start and stop codons), moderate impact (non-synonymous variations), low impact (synonymous variations in coding regions, start and stop codons), and modifier impact (in the non-coding region) (Fig. 3**A**). Among all SNPs, modifier SNPs were the most abundant, followed by moderate, low, and high-impact SNPs. The share of modifier InDels was also the highest among all InDels and the second most abundant type was moderate-impact InDels, but the number of high-impact InDels was higher than low-impact InDels (Fig. 3**A**) (**Supplementary Table S1**). The high and moderate impact SNPs and InDels were further allotted in missense (introduce amino acid substitutions), nonsense mutations (change amino acids specifying codons into stop codons), and silent categories, of which variants causing missense mutations were the highest in number (Fig. 3**B**).

Besides, the functional classification of the variety-specific SNPs and InDels signified the presence of a greater percentage of the high impact SNPs in acc. 2015 (0.1 %) than in O9897 (0.09 %) (**Supplementary Table S1**). Again, high impact InDel variants were observed in a greater percentage (0.41 %) in both plants compared to the SNPs (**Supplementary Table S1**). This finding complies with the fact that InDels have more contributions in sequence diversity[48] with a greater effect on protein structure and function than SNPs[49] which may serve as a potential marker linked to cold-tolerant traits in genetic engineering and breeding.

3.4. Gene ontology and enrichment analysis of identified SNPs and InDels

3.4.1. Gene ontology and enrichment analysis of identified SNPs

GO analysis showed that target genes implicated in the term "Response to stress (GO:0006950)" were peroxidase (COLO4_03296), ER lumen protein retaining receptor (COLO4_08646), and hexosyltransferase (COLO4_24192) for acc. 2015-specific SNPs. Among them, peroxidase has been reported to be responsible for the low-temperature tolerance in plants.[50] In the case of the sensitive species O9897, no SNPs were found in the genes of "Response to stress (GO:0006950)" pathways. This genomic variation may have some role in low-temperature tolerance for the tolerant jute variety acc. 2015 in comparison to the sensitive variety O9897.

Pathway enrichment analysis was carried out for genes related to (1) SNPs specific to the two jute varieties, (2) InDels specific to the two jute varieties. Enrichment was performed on three categories of GO terms: Biological Process, Cellular Component, and Molecular Function. GO enrichment analysis of these genes produced several enriched terms for O9897 and acc. 2015, considering a 10 %FDR threshold for significance. Table 2 represents the GO terms that are the most highly enriched within the provided gene list.

It was observed that the gene sets harboring O9897 specific SNPs are the most enriched in biological processes related to transcription regulation (KW-0805); cellular components related to nucleus (GO:0005634), and molecular functions related to DNA binding (GO:0003677). Genes associated with these terms included several cold-responsive related transcription factors like AP2/ERF domain containing protein, BHLH domain containing protein, HTH myb type domain containing protein, Auxin-responsive protein, and High mobility group containing protein. AP2/ERF domain-containing protein activates the expression of abiotic stress-responsive genes via specific binding to the dehydration-responsive element/C-repeat (DRE/CRT) *cis*-acting element in their promoters[51]. BHLH domain-containing protein controls the CBF class of proteins that are responsible for cold

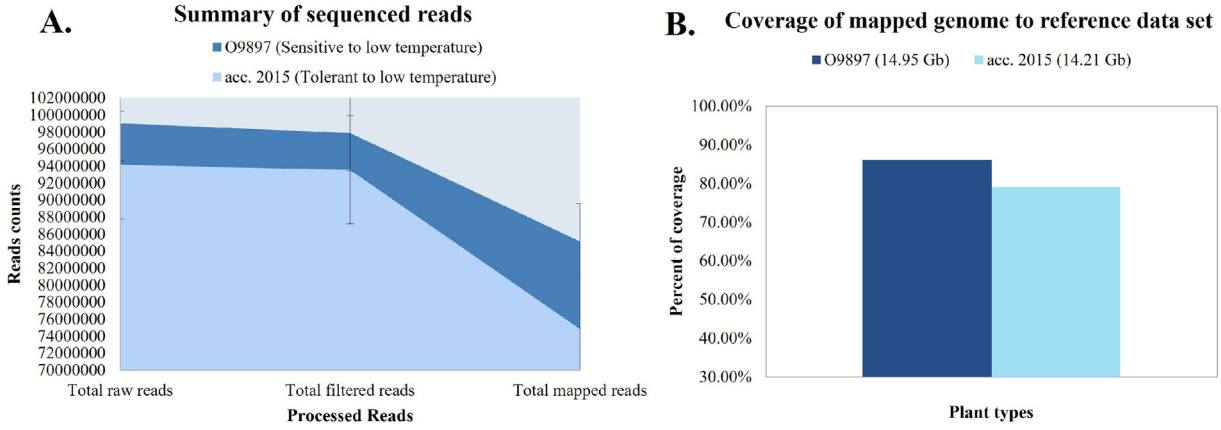

Fig. 1. Total sequence read information for *C. olitorius* var. O9897 (sensitive) and acc. 2015 (tolerant). **A.** Summary of the sequenced reads, **B.** Coverage of the mapped genome to the reference genome.

Table 1
Comparison of genomic variations in *C. olitorius* low-temperature sensitive var. O9897 and resistant variety acc. 2015.

Features	O9897	acc. 2015
Total raw reads	99,031,906	94,144,626
Total filtered reads	97,873,202	93,562,924
Number of bases (Gb)	14.95	14.21
Total mapped reads	85,149,009 (86.11 %)	74,844,186 (79.01 %)
SNPs	1,073,873	990,413
InDel	513,682	489,749
InDel/SNP ratio	0.47	0.49
SNP Het/Hom ratio	5.97	4.30
InDel Het/Hom ratio	1.63	1.42
SNPs Transitions/Transversions	1.91	1.87
Dominant mutation	C to T	C to T

stress and freezing temperature tolerance in plants[52], HTH myb-type domain-containing proteins are involved in osmotic stress signal perception regulating chill and drought stress.[53] Auxin-responsive protein regulates plant growth[54] and high mobility group containing protein functions in cold stress response Mallik et al., 2020;1863[55]: 194644. Single nucleotide changes in these genes may alter their function and could be a contributory factor to the sensitivity of O9897 to low-temperature stress especially since no such SNPs were found in the tolerant jute plant.

In addition, genes harboring acc. 2015 specific SNPs are enriched in biological processes related to carbohydrate metabolic process (GO:0005975); cellular components related to nucleosome (GO:000786), and molecular functions related to protein heterodimerization activity (GO:0046982), cysteine-type peptidase activity (GO:0008234). Besides, several studies of temperature-induced changes in plants demonstrated alteration in the biological process "carbohydrate metabolism" in response to cold stress resulting in an increase in the content of soluble sugars to ensure membrane integrity of the plant cells.[56] In *Arabidopsis*, members of cysteine-type peptidase family have been reported to play several roles as regulators of plant development and disease resistance.[57] Heterodimerization of MdMYC2 with MdCIbHLH1 was found responsible in apples for increasing transcriptional activity levels to confer cold tolerance[58]. Moreover, SNPs were reported to be involved in the alternative function to confer cold tolerance in rice[59] and as important markers for mapping candidate genes.[60]

3.4.2. Gene ontology and enrichment analysis of identified InDels

Gene enrichment analysis of the plant-specific InDels revealed the O9897-specific InDels to be most enriched in biological processes related to transport (GO:0006810) (Table 2**)** whereas acc. 2015 specific InDels were enriched in biological processes related to organonitrogen compound metabolic process (GO:1901564) and gene expression (GO:0010467). At the molecular level, low-temperature stress can cause alteration in the fluidity of a plant cell membrane and consequently in membrane structure[61]. Besides, the accumulation of toxic compounds, solute leakage, and dehydration are also responsible for cell rupture in a low-temperature sensitive plant.[61] Hence, the presence of InDels in genes related to transport may cause cold stress-related pathogenesis and may likely be a phenomenon in the low-temperature sensitive variety O9897. On the contrary, the presence of specific InDels unique to the acc. 2015 variety within genes related to metabolic process and gene expression regulation might indicate a change in the plant's metabolic characteristics,[62] potentially contributing to its tolerance.

3.5. Copy number variation analysis in O9897 and acc. 2015

Analysis of copy number variation by Delly revealed copy number changes in 3674 positions in O9897 and 3576 positions in acc. 2015 of which 2538 were shared CNVs (Fig. 4**A**). Copy number profiles of the two varieties were also analyzed using Control-FREEC in which O9897 was used as control and acc. 2015 was used as the sample. Firstly, Control-FREEC generated raw copy number profiles for both plant types and then predicted copy number alterations in tolerant sample control-sensitive acc. 2015 against the controlsensitive plant O9897. From that profile, copy number alterations were found to be present in 675 positions in acc. 2015 in comparison to O9897. Among them, 85 copy number variation (CNV) positions were identified by both tools (Fig. 4**A**) and distinguishable in acc. 2015 compared to O9897. About 44 contigs were found to possess multiple copy numbers with a gain of function of 144 loci in *C. olitorius* acc. 2015 (Fig. 4**B**) but were absent in the sensitive verity O9897. According to the gene enrichment analysis, genes present in these loci (120 gene loci) **(Supplementary Table S2)** are mostly involved in stress-responsive pathway, signaling pathway, transporting proteins, metabolic pathways which are concomitantly activated in response to cold stress, and function in cold stress acclimation.

Besides, the identified 85 distinctive copy number variation regions in acc. 2015 corresponding to 60 genes **(Supplementary Table S3)** were categorized into different pathways using KAAS (KEGG Automatic Annotation Server) resulting in the assignment of 23 KEGG IDs (Table 3). Most of these genes were found to be associated with signal transduction and the rest were related to amino acid metabolism, lipid metabolism, membrane transport, metabolism of

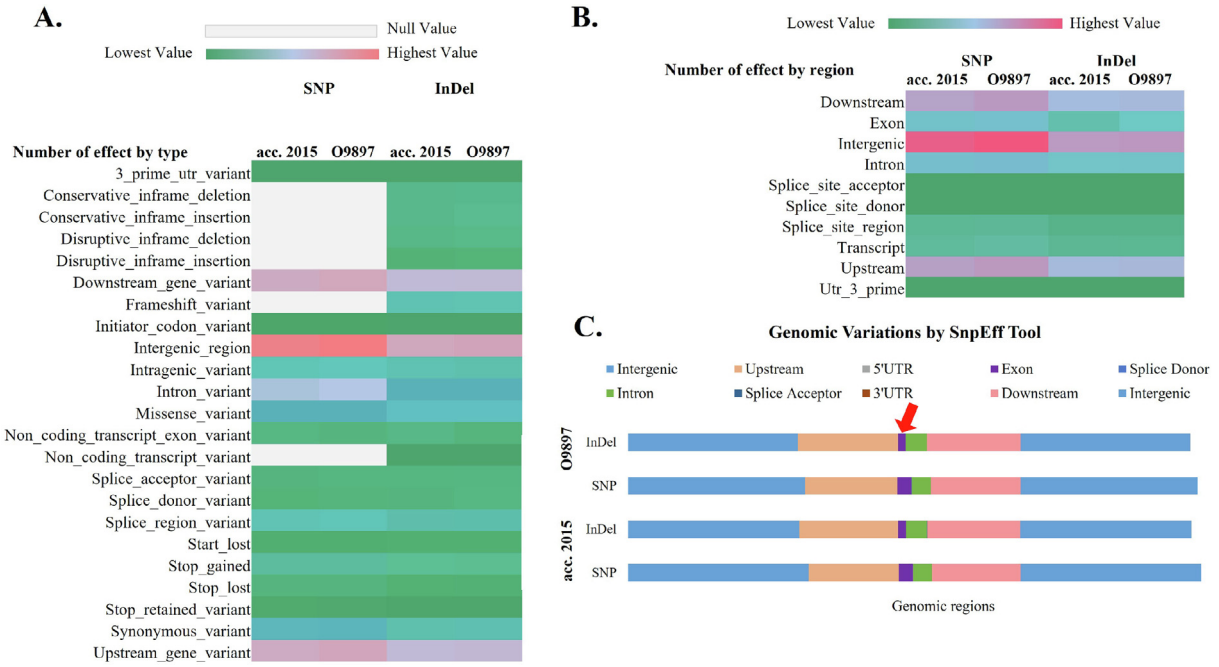

Fig. 2. Genomic variations by different types of SNPs and InDels in two plants *C. olitorius* low-temperature sensitive variety O9897 and resistant variety acc. 2015. **A.** Heat map representing the number of effects by types of SNPs and InDels (Green color represents the lowest number of SNPs or InDels types, red color represents the highest number of SNPs or InDels by this type and gray color represents no SNPs or Indels present by this type). **B.** Heat map showing the number of effects by region of SNPs and InDels (Green color represents the lowest number of SNPs or InDels types and red color represents the highest number of SNPs or InDels by this type). **C.** Schematic representation of genomic variations by SNPs and InDels (different colors represent the different parts of the genome and the area in the bar shows the abundance of the SNPs and InDels by that type, four different bars indicate the four sets of samples: O9897 InDels and SNPs, acc. 2015 InDels and SNPs, the red arrow indicating the exonic region in four sets of samples). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

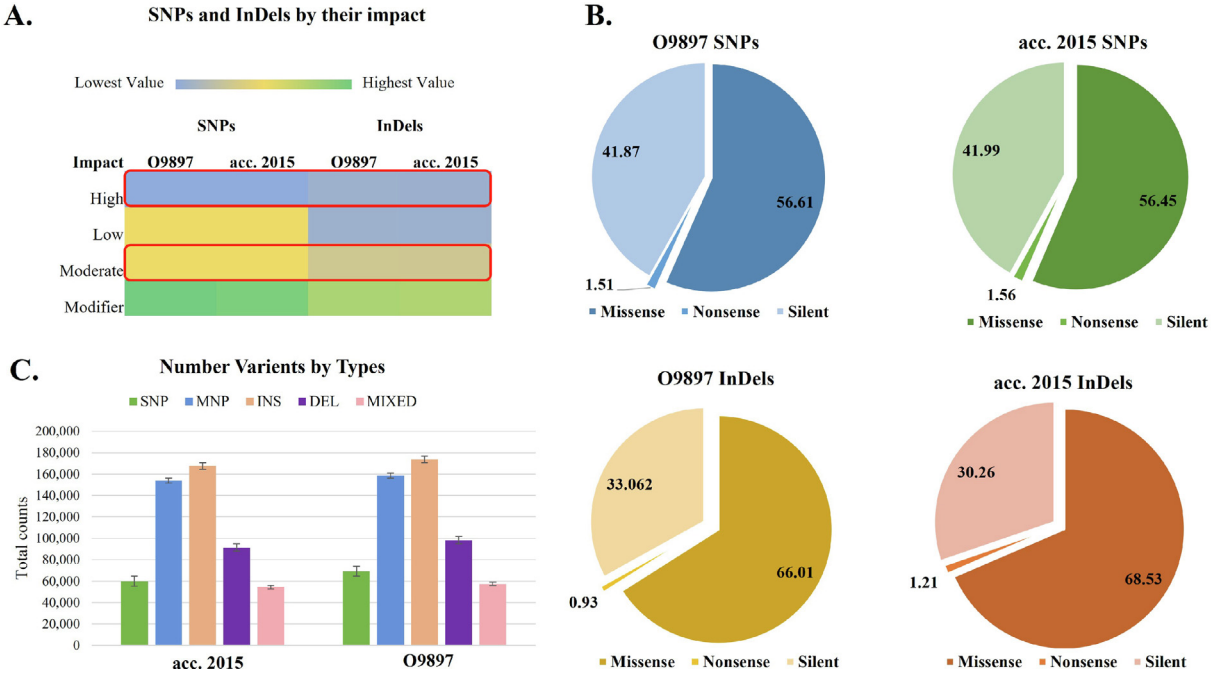

Fig. 3. Distribution of SNPs and InDels by functional class present in two jute plants *C. olitorius* O9897 and acc. 2015. **A.** Distribution of SNPs and InDels by their impact (Blue color shows the lowest frequencies, Green color shows the highest frequencies, and Red rectangular indicates the SNPs and InDels which further categories according to the amino acid changes). **B.** Abundance of high and moderate impact SNPs and InDels according to amino acid changes (alternative amino acid substitution: missense, no amino acid coding: nonsense, no change in amino acid: silent). **C.** Number of total variants present in InDel dataset (different colors represent different types, Insertion causes the maximum variants in InDel polymorphism which is followed by MNP, Deletion, SNPs, and mixed). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2
Results of GO enrichment analysis SNPs and InDels specific genes' set in two types of plants.

Category	SNPs specific to the two jute varieties			
	O9897		acc. 2015	
	GO annotation	Genes involved	GO annotation	Genes involved
Biological Process	Transcription regulation (KW-0805)	1. AP2/ERF domain containing protein	Carbohydrate metabolic process (GO:0005975) Response to stress (GO:0006950)	1. Histone H2B 2. Histone H2A
Cellular Component	Nucleus (GO:0005634)	2. BHLH domain containing protein	Nucleosome (GO:0000786)	3. Histone H4
Molecular Function	DNA-binding (GO:0003677)	3. HTH myb type domain 4. Auxin responsive protein 5. High mobility group containing protein	Protein hetero-dimerization activity (GO:0046982), Cysteine-type peptidase activity (GO:0008234)	4. Chitinase 5. Glycoside hydrolase, family 28 6. Peroxidase
Category	**InDels specific to the two jute varieties**			
	O9897		**acc. 2015**	
	GO annotation	Genes involved	GO annotation	Genes involved
Biological Process	Transport (GO:0006810)	1. Acyltransferase 3 2. Terpenoid cyclases/protein prenyltransferase	Organonitrogen compound metabolic process (GO:1901564) Gene expression (GO:0010467)	1. ATP binding domain containing protein
Cellular Component	Integral component of membrane (GO:0016020)	3. Formate/nitrite transporter 4. Phosphotransferase system, EIIC	Cytoplasm (GO:0005737)	2. Ribosomal protein 3. Putative ABC transporter
Molecular Function	Transferase activity (GO:0016740), catalytic activity (GO:0003824)	5. Binding-protein-dependent transport systems inner membrane component 6. White-brown-complex ABC transporter family	ATP binding (GO:0005524)	4. ATP binding protein

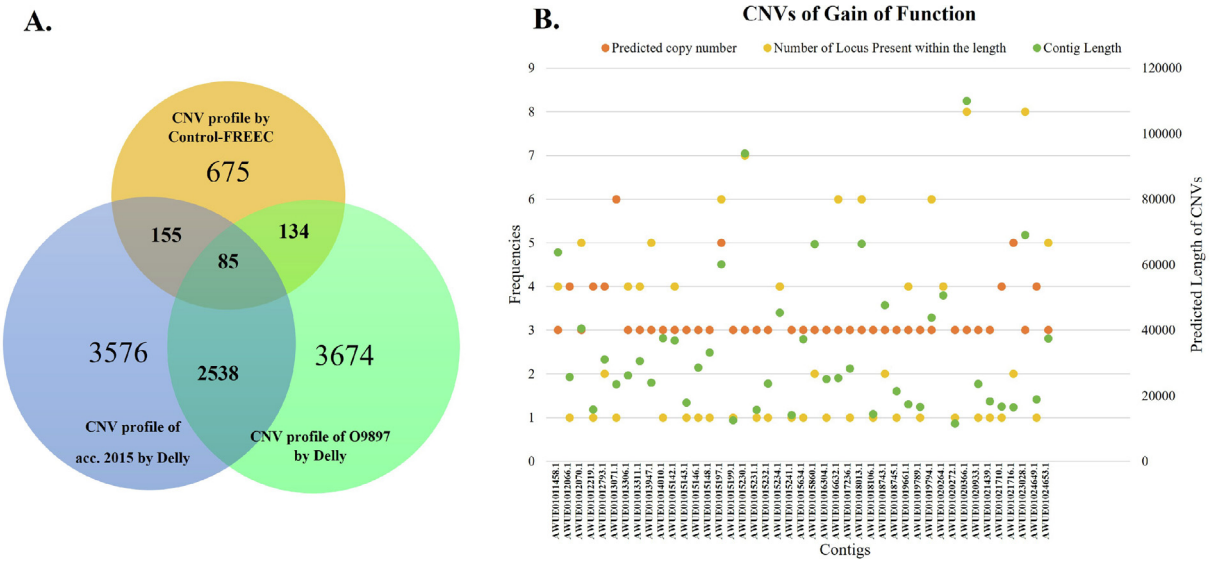

Fig. 4. Copy number variation analysis using Delly and Control-FREEC. **A.** Venn diagram representing 85 CNVs observed by both tools **B.** CNVs of gain functions and the frequencies of their copy numbers and gene locus in acc. 2015 predicted by Control-FREEC tools.

terpenoids, polyketides, and other secondary metabolites, and genetic information processing.

Proteins present in these variant regions are known to mediate alternative physiological changes in tolerant plants through a variety of mechanisms to gain the adaptive cold tolerance features which include alternative cell structure, cell and ion homeostasis, osmotic balance, cold adaptive metabolism, membrane transport, effective tissue arrangement and organogenesis[63–64]. Functional gain in these gene loci may aid the tolerant species in acquiring effective cellular and metabolic profiles by providing sufficient alternative functional proteins to be activated under low-temperature stress[65]. The alternative gain of function loci includes S-locus glycoproteins which induce seed germination and break the dormancy in response to low temperature and activate the concomitant cold tolerant gene network[65]. The CNVRs leading to a possible gain of function also possessed the helicase-like proteins that are known as early regulatory factors of freezing and chilling tolerance.[66–67] We found Myb-like transcription factors to be present in the CNVRs leading to a gain of function which attributes to significant involvement in cold tolerance induction through a variety of pathways from regulation of CBF genes to cell development, organogenesis, secondary metabolite production in response to cold acclimation process[68]. The CNVRs also contained CBL-interacting serine/threonine-protein kinase 16 which has been reported to be functional in different stress responses and development[69], In addition, these CNVRs include genes encoding different regulatory proteins that were reported to be involved in the regulation of replication, transcription,

Table 3
Result of pathway analysis by KAAS for genes present in CNVRs.

Protein ID	Protein names	KEGG ID	Pathway
A0A1R3KM80	Phospho-2-dehydro-3-deoxyheptonate aldolase (EC 2.5.1.54)	K01626	09,105 Amino acid metabolism
A0A1R3KM84	ERCC4 domain-containing protein	K10848	09,120 Genetic Information Processing
A0A1R3KMC9	Protein kinase domain-containing protein	K14498	09,132 Signal transduction
A0A1R3KMB9	Recoverin	K06268	09,132 Signal transduction
A0A1R3KM86	Decapping nuclease (EC 3.6.1.-)	K14845	09,182 Protein families: genetic information processing
A0A1R3KAG4	EGF-like domain-containing protein	K03234	09,132 Signal transduction
A0A1R3KA54	3-isopropylmalate dehydrogenase (EC 1.1.1.85)	K00052	09,105 Amino acid metabolism
A0A1R3JVF1	Retrotran_gag_3 domain-containing protein	K10901	09,120 Genetic Information Processing
A0A1R3J5B5	SAM dependent carboxyl methyltransferase	K21482	09,191 Unclassified: metabolism
A0A1R3J5B3	Appr-1-p processing	K23518	09,191 Unclassified: metabolism
A0A1R3J5D2	Appr-1-p processing	K23518	09,191 Unclassified: metabolism
A0A1R3ISC9	AMP-dependent synthetase/ligase	K10526	09,103 Lipid metabolism
A0A1R3IKX8	LRRNT_2 domain-containing protein	K13420	09,132 Signal transduction
A0A1R3HKW0	ABC transporter B family member 4-like	K05658	09,131 Membrane transport
A0A1R3HEH5	RING-type E3 ubiquitin transferase (EC 2.3.2.27)	K16275	09,182 Protein families: genetic information processing
A0A1R3HEB5	GST N-terminal domain-containing protein	K01800	09,105 Amino acid metabolism
A0A1R3HEC6	TFIIS N-terminal domain-containing protein	K17498	09,182 Protein families: genetic information processing
A0A1R3GT35	Annexin	K17098	09,193 Unclassified: signaling and cellular processes
A0A1R3GTA0	LRRNT_2 domain-containing protein	K13466	09,159 Environmental adaptation
A0A1R3FWG4	Mitochondrial substrate/solute carrier	K15113	09,183 Protein families: signaling and cellular processes
A0A1R3FWG1	BTB/POZ-like protein	K10523	09,132 Signal transduction
A0A1R3FWG3	BTB/POZ-like protein	K10523	09,132 Signal transduction
A0A1R3FWM4	Cytochrome P450	K04123	09,109 Metabolism of terpenoids and polyketides

translation, and cell cycle when upregulated or alternatively expressed such as cyclins[70], G-patch domain-containing protein[71], DUF4283 domain-containing protein[72], F-box associated domain-containing protein[73], RRM domain-containing protein[74], cyclin PHO80-like protein[75] and putative phosphoprotein phosphatase.[76]

Again, in this study, some regulatory proteins were present in the CNVRs which were found to be upregulated in previous studies in response to cold stress to provide effective functions. These adaptive functions exert cell shape and microtubule dynamics, cell polarity and cytokinesis, microtubule organization, organelle distribution, vesicle transport, and cell growth through upregulating the function of different proteins which include cation-transporting P-type ATPase[77], targeting protein for Xklp2 containing protein[78], TIR domain-containing protein[78], RRM domain-containing protein[74], kinesin motor domain-containing protein[79], protein kinase domain-containing protein[80], leucine-rich repeat-containing N-terminal plant-type domain-containing protein[81], putative DNA topoisomerase I (ISS) protein[82], ENT domain-containing protein BTB/POZ-like proteins[83]. Moreover, the CNVRs of gain of function possess a number of effector proteins including potassium transporter 5 maintaining ionic balance osmotic pressure and cell homeostasis when upregulated in tolerant plant types[84–85]. laccase-17-like protein highly expressed in cold temperature results in lignification-led adaptation,[86–87] annexin expression in stress responses confer cell growth and development[88], acyl carrier protein 4 involved in transformation of fatty acid lead to chilling tolerance[89], endoplasmic reticulum vesicle transporter protein-mediated regulation of lipid translocation and composition upon cold stress and abundance of the genes maintain membrane fluidity and viscosity with regard to the cold acclimation[90–91]. Although the tolerant genome does not contain the CNVs in frequently reported CBF/DREB pathway[92] regulated by MYB transcription factor, a positive regulator helicase-like protein locus was found to have high copy number with a gain of function that was reported to be involved in cold-induced adaptation in a previous study[93]. Besides, tolerant variety acc. 2015 contained a high copy number of retrotransposon Copia-like N-terminal domain-containing proteins which modulate chromosome structure, gene expression, and regulation, as well as adaptation and evolution, were found to have high copy numbers and expressed with defense-related genes when induced by wound or stress.[94–95] According to this comparative genomic study, we found potential genetic variances (SNPs, InDels, CNVs) that are linked to the important gene loci responsible for cold-tolerant induced adaptive features in plants. These genetic variances can be applied as markers in search of putative traits for example defense genes, genes involved in maintaining homeostasis, germination that need to be introduced in the genetically engineered cold-resistant jute as well as other plants through marker-assisted breeding and genetic engineering.

4. Conclusion

Low temperature is a major abiotic stress responsible for hampering plant growth and development. Adaptation to this stress is a complex process that comprises several genes, transcription factors, metabolites, and other cellular changes. To unveil the mechanism of these pathways, the identification of appropriate molecular markers associated with the respective genes is nascent to the development of tolerant jute plant species. Our study identified SNPs and InDels in low-temperature stress-inducible genes, potentially causing stress-related pathogenesis in sensitive jute plants but not in tolerant ones. Again, some SNPs and InDels were found to be present in the tolerant jute plant gene loci that are documented to be responsible for resistant phenotypes. High copy numbers with a gain of function were observed in genes of the tolerant jute accession in cold stress-responsive regulatory proteins and transcription factors as well as their downstream effector proteins that confer adaptive phenotypic features including lignification, alternative cell wall remodeling, cytoskeleton mobility, development, and growth which were absent in sensitive plant type. The findings of this study are expected to be a starting point in developing low-temperature tolerant cultivars to improve jute agriculture capable of growing around the year in a tropical country. Besides, this study will encourage other researchers and breeders to perform the resequencing of more jute accessions, to discover many other valuable agronomic traits.

Availability of data and material

The datasets (whole genome resequencing data of the *C. olitorius* resistant variety accession no. acc. 2015 and sensitive variety O9897) generated during the current study are available in the NCBI Sequence Read Archive (SRA) repository, under the Bio-Project (https://www.ncbi.nlm.nih.gov/bioproject/) accession ID PRJNA951086.

CRediT authorship contribution statement

Athoi Ganguly: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Formal analysis, Data curation, Conceptualization. **Shaheena Amin:** Writing – review & editing, Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Al-Amin:** . **Farhana Tasnim Chowdhury:** Project administration, Methodology, Investigation, Conceptualization. **Haseena Khan:** Writing – review & editing, Supervision, Project administration, Investigation, Funding acquisition, Conceptualization. **Mohammad Riazul Islam:** Writing – review & editing, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization.

Data availability

Bio-Project accession ID PRJN951086. (Original data) (NCBI SRA)

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors concede the support of Bangladesh Research and Education Network (BdREN) for their server support.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jgeb.2024.100376.

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