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# IDENTIFICATION OF CELL WALL INVERTASE ACTIVITIES IN SELECTED WHEAT CULTIVARS FROM SINDH FOR CROP IMPROVEMENT

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**ABSTRACT**

CWI markers, also known as Cell Wall Invertase markers, play a pivotal role in wheat breeding and crop improvement. These markers help identify wheat varieties with desirable traits related to yield potential, stress tolerance, drought tolerance, and overall crop performance. The CWI gene, found in bread wheat, has been identified on chromosomes 4A, 5B, and 5D, all of which contain the conserved WECPDF domain. The main role of the Cell wall invertase (CWI) gene is to hydrolyze sucrose into glucose and fructose. Harnessing the power of CWI markers contributes to the advancement of sustainable agriculture practices and the development of high-performing wheat varieties to meet the demands of diverse agricultural landscapes. In this study, we investigated the prevalence of CWI markers, specifically CWi21 and CWi22, in wheat varieties cultivated in Sindh, Pakistan. We collected a set of thirteen wheat genotypes cultivated in the Sindh province for the screening of Cell Wall Invertase markers through polymerase chain reactions. Our analysis revealed the diversity of the markers CWi21 and CWi22 in wheat genotypes. The marker CWi21 was present in 30% of the wheat varieties, while CWi22 was detected in 70% of the wheat varieties.

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## INTRODUCTION

Wheat (*Triticum aestivum* L.) holds significant importance in the Poaceae family as a widely cultivated crop, primarily utilized by humans in various forms like chapatti, bread, cookies, biscuits, cakes, and other baked goods. It serves as a vital energy source, providing 73% of calories, 7% carbohydrates, 6% dietary fiber, 12% water, 2% fats, 6.2% proteins, and 1.8% minerals. Wheat straw, on the other hand, is employed as animal feed, contributing to increased milk production in cattle, and is also utilized as a roofing material in rural areas, enhancing plaster hardness and toughness (Debasis and Khurana, 2001).

Wheat crops exhibit adaptability to both summer and winter conditions, enabling year-round cultivation in many countries, making it the second-largest cultivated cereal crop globally, following rice. Pakistan, primarily an agricultural nation, relies heavily on the economic contributions of agriculture, with Sindh and Punjab Provinces playing key roles. Sindh covers a land area of 1,120,300 hectares, yielding 18,237,600 tons of wheat, where Sindh's share is 26,24,900 tons. The average wheat production in Pakistan is 54.9 maunds (2196 Kg) per hectare, with the Sindh province recording an average yield of 58.575 maunds (2343 Kg) per hectare (Economic Survey of Pakistan, 2013).

The Cell Wall Invertase (CWI) enzyme plays an important role in plants, particularly in wheat. Invertase, discovered by Crispeels in 1990, converts sucrose to

glucose and fructose. Among the types of invertase, Cell Wall Invertase (CWI) is highly associated with kernel weight and plays a crucial role in sink tissue synthesis and carbon partitioning in the kernel (Villreal et al., 1997, 1998). The enzyme's significance is highlighted in determining wheat crop yield potential, with specific markers, CWi21 and CWi22, associated with the TaCWI-A1 locus on chromosome 2A (Arora et al., 2019), offering potential applications in wheat breeding for improved grain yield (Ma et al., 2012). Cell Wall Invertase (CWi), a key enzyme influencing sink tissue growth, demonstrates a strong correlation with kernel weight (Yang et al., 2004). Recently, the complete genomic DNA sequence of the CWi gene located on the 2A chromosome in wheat has been disclosed. The presence of CWi in a crop notably impacts kernel weight and size. Molecular markers for the CWi21 and CWi22 loci, regulating low and high kernel weight, have been developed, presenting a novel tool of considerable significance in crop science for identifying wheat genotypes with elevated kernel weight.

In the realm of plant physiology, assimilated carbon, predominantly sucrose is transported from photosynthetic tissues (source) to non-photosynthetic tissues (sink) (Zhang et al., 2022). Higher plants possess three types of invertase: cytoplasmic invertase, vacuolar invertase, and Cell Wall Invertase (CWI). CWI, in particular, has a close association with kernel weight, playing a pivotal role in synthesizing sink tissue and coordinating carbon partitioning in the kernel (Liang et al., 2001). The intimate connection between grain yield in wheat and both kernel number per unit area and kernel weight, CWI assumes a crucial role in determining the yield potential of wheat crops. During drought conditions, cell wall invertase activity becomes particularly crucial for plants. As water becomes scarce, plants experience reduced turgor pressure and compromised cellular functions. However, cell wall invertase plays a pivotal role in this scenario by breaking down stored sucrose into glucose and fructose. This breakdown provides the necessary energy and carbon sources for essential metabolic processes, aiding in osmotic adjustment and the synthesis of protective compounds. By maintaining osmotic balance through the breakdown of sucrose, cell wall invertase helps plants sustain cell turgor and integrity despite water scarcity (Li et al., 2021). Insights from studies like those by Mujeeb-Kazi (1982) and Khodadadi et al. (2011) contribute to understanding potential sources of variation applicable in wheat breeding programs. Various approaches, encompassing both morphological and molecular methods, are employed to probe genetic diversity in wheat. The objectives of the study involve evaluating wheat varieties to assess molecular diversity, especially using the CWI markers, to pinpoint lines with high kernel weight for wheat improvement. The exploration of genetic diversity in adapted cultivars holds substantial promise for crop improvement. Information from such studies proves valuable for germplasm management and genotype selection in breeding programs.

## MATERIALS AND METHODS

### Wheat germplasm

A set of thirteen local wheat varieties, collected from the Nuclear Institute of Agriculture (NIA) Tando Jam and the Wheat Research Institute (WRI) Sakrand, was used for the identification of the Cell Wall Invertase (CWi) marker. Details of the varieties are given in Table 1.

### DNA extraction

The total genomic deoxyribonucleic acid (DNA) was extracted from individual wheat genotypes using the CTAB method (Cetyltrimethylammonium bromide), as outlined by Doyle (1990) with minor adjustments. To initiate the DNA extraction process, small wheat plants (one week old) were utilized. The wheat genotypes were cultivated in pots, and after two weeks, the seedlings were harvested. Subsequently, the leaves were ground using a pestle and mortar. In the resulting mixture, 500 µl of CTAB solution was added. Upon achieving a blackish-green solution, the mixture was transferred to a 500 µl tube and centrifuged at 5000 rpm for 5 min. The supernatant was discarded, and the pellet was then transferred to a new Eppendorf tube. To this, 70 µl of isoamyl alcohol was added, and the solution was left exposed to air at normal room temperature for 1 h.

### DNA quantification

DNA purity was monitored by using agarose gel (1.5%) under ultraviolet (UV) light after staining with ethidium bromide. PCR amplification reactions were conducted in 25 µl reaction mixtures, comprising 50-100 ng of total genomic DNA, 0.25 mM of each primer, 200 mM of each dGTP, dATP, dTTP, and dCTP, 10 mM Tris, 50 mM KCl, 2.5 units of Taq DNA polymerase, and 1.5 mM MgCl2 (Dweikat et al., 1993). The amplification conditions were as follows: an initial denaturation step for 1 min at 94°C,

followed by 35 cycles, each consisting of a denaturation step for 1 min at 94°C, an annealing step for 1 min (with some modification in annealing temperature, set at 56°C, 60°C, and 62°C for optimizing different markers), and an additional step for 2 min at 72°C. Following the last cycle, a 7-minute extension step at 72°C was implemented to ensure the completion of the primer extension reaction.

**Primers for PCR**

The detail of molecular markers used in primers for identifications of CWi genes in wheat genotypes is given in Table 2.

**Electrophoresis**

The PCR products were determined by loading 10 µl of the PCR product onto 1.2% agarose gels in 1X TBE buffer and visualizing them under ultraviolet (UV) light after ethidium bromide staining. A Gel documentation system (Digecel) was used to observe the absence or presence of the gene (Doyle, 1990).

**Table 1: Details of wheat varieties assessed for cell wall invertase activity.**

| Sr. No. | Variety | Parentage |
|---|---|---|
| 1 | Anmol 91 | KVZ/ TRM //PIM /ANA |
| 2 | Skd- 1 | HD-2329 PAU-ACC-3079 |
| 3 | Mehran | VEERY 5'S'CM33027-F-15M-500Y-OM-57B-OY |
| 4 | Abadgar 93 | YAKTANA54 X NORIN 10-BREVOR X SON 64 |
| 5 | TJ 83 | TZPP-PL X 7C |
| 6 | Td 1 | MAI 'S' x NORTEMO 65 x H68 |
| 7 | Moomal 2002 | BUS'S'/4/ TZPP// 1RN46/ CN 067/ 3/PRI-FIK 5644 |
| 8 | Sassui | CHIL/ ALD// PVN/ Yacora-70 |
| 9 | Sarsabz | PI-FROND/PI-MAZOEs |
| 10 | Bhitai | VEE/TRAP#1//SOGHAT-90 |
| 11 | Pavon | VCM x CNO 'S'-7C/KAL-BB |
| 12 | WL-711 | S308-CHR/KAL |
| 13 | Bakhtawar93 | JUP/BJY'S//URES |

Table 2: Detail of molecular markers used in Primers for identifications of CWi genes in wheat genotypes.

| Sr. No. | MARKER | Gene | Sequence | Bp Linked | Reference |
|---|---|---|---|---|---|
| 1 | Cwi21-F | CWi21 | GTGGTGATGAGTTCATGGTTAAG | 404 | Ma et al. 2012 |
| | Cwi21-R | | AGAAGCCCAACATTAAATCAAC | | |
| 2 | Cwi22-F | CWi22 | GGTGATGAGTTCATGGTTAT | 402 | Ma et al. 2012 |
| | Cwi22-R | | AGAAGCCCAACATTAAATCAAC | | |

**RESULTS AND DISCUSSION**

Data were compiled for the absence and presence of markers linked to the Cell Wall Invertase (CWI) gene (Table 3). The data were subjected to frequency distribution, and graphs were plotted. The CWi-22 marker was identified in 9 (70%) cultivars (Anmol 91, Skd-1, TD-1 11-12, and WL-711), while the marker was absent in the remaining 30% of genotypes (Mehran 89, Abadgar, TJ 83, Moomal, Sassui, Sarsabz, Bhitai, Pavon, and Bakhtawar). Details of molecular markers linked to the CWi 21 locus were given in Figure 1.

The CWi-21 marker was identified in 4 out of 13 cultivars (Anmol 91, Skd-1, TD-1 11-12, and WL-711), accounting for 30% of the total cultivars. Conversely, the marker was absent in the remaining nine (Mehran 89, Abadgar, TJ 83, Moomal, Sassui, Sarsabz, Bhitai, Pavon, and Bakhtawar), genotypes representing 70% of the genotypes. Details regarding Gel Electrophoresis for molecular markers linked to the CWi-22 locus was provided in Figure 2. Frequency distribution showing presence of CWI21 and CWI22 markers in wheat genotypes in Figure 3.

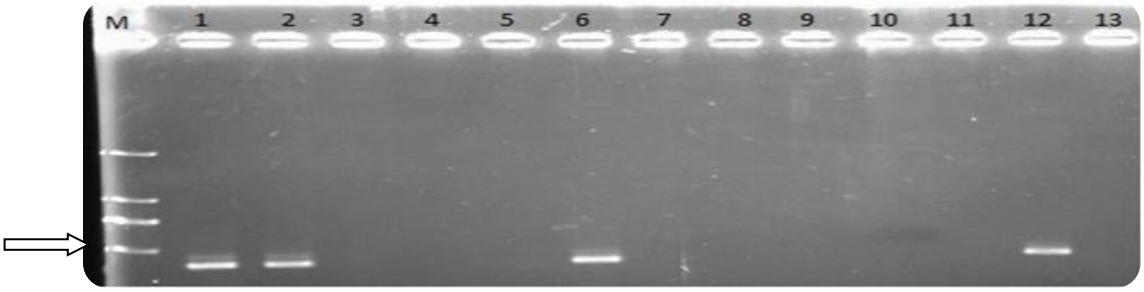


Figure 1: Agarose gel electrophoresis of 13 wheat varieties for identification of CWi 21 marker (bp 404).

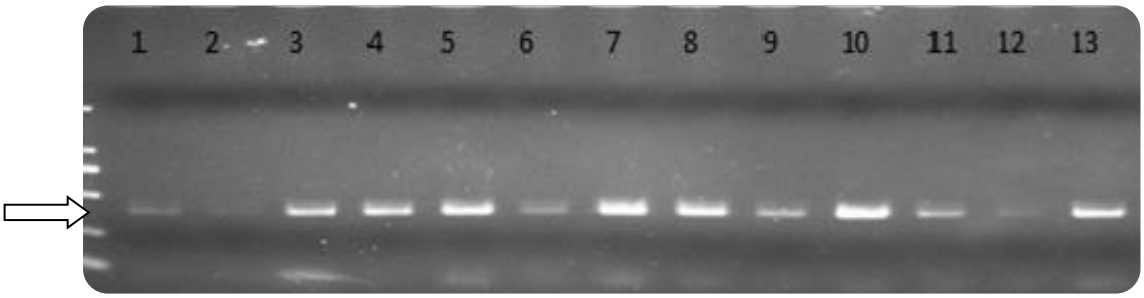


Figure 2 Agarose gel electrophoresis of 13 wheat varieties for identification of CWi 22 marker (bp 402).

Table 3: Details of presence and absence of CWi21 and CWi22 markers in wheat genotypes.

| Sr. No. | Accessions | Cwi-21 | Cwi-22 |
|---|---|---|---|
| 1 | Anmol 91 | Present | Absent |
| 2 | Skd-1 | Present | Absent |
| 3 | Mehran 89 | Absent | Present |
| 4 | Abadgar | Absent | Present |
| 5 | TJ 83 | Absent | Present |
| 6 | Td-1 11-12 | Present | Absent |
| 7 | Moomal | Absent | Present |
| 8 | Sassui | Absent | Present |
| 9 | Sarsabz | Absent | Present |
| 10 | Bhitai | Absent | Present |
| 11 | Pavon | Absent | Present |
| 12 | WL-711 | Present | Absent |
| 13 | Bakhtawar | Absent | Present |

Molecular markers prove to be more dependable and less influenced by environmental factors compared to morphological traits. In this study, alongside agro-morphological characterization, markers were employed to identify CWI genes in wheat cultivars associated with both high and low grain weight. The findings from the marker analysis indicated that CWi21 was identified in four varieties viz. Anmol 91, Skd-1, Td-1 11-12, and WL-711, each exhibiting a 404-bp fragment. A similar investigation conducted by Ma et al. (2012) reported the presence of CWi genes in a diverse collection of wheat cultivars. When aligning the results of yield data with molecular markers, the grain data from the field correlated with the marker data. For instance, CWi21, linked to high kernel weight, was detected in Anmole (with high kernel weight of 5.88 g), SKd 1 (with high kernel weight of 5.83 g), Td 1-11-12 (with high kernel weight of 5.53 g), and WL-711 (with high kernel weight of 5.41 g). Conversely, the CWi22 fragment, associated with low kernel weight, was identified in nine varieties (Mehran 89, Abadgar, TJ 83, Moomal, Sassui, Sarsabz, Bhitai, Pavon, and Bakhtawar)

with a 402-bp fragment. These varieties also exhibited lower grain weight under field conditions, consistent with similar observations reported by Ma et al. (2012). In this study, the prevalence of CWI markers, CW21 and CW22, in wheat varieties cultivated in Sindh, Pakistan, was investigated, revealing their significant distribution among the studied varieties. The identification of these markers holds immense importance for wheat breeding programs, as they are associated with crucial agronomic traits such as yield potential and stress tolerance. CWI markers, particularly CW21 and CW22, have been linked to enhanced sucrose metabolism, which influences various physiological processes crucial for wheat growth and development (Guo et al., 2014). Additionally, these markers have been implicated in regulating grain filling and starch accumulation, thereby impacting yield and quality attributes (Barrero et al., 2013). By elucidating the prevalence of CWI markers in Sindh's wheat varieties, this study provides breeders with valuable information for selecting parental lines with desirable traits for future breeding programs. Moreover, policymakers can utilize these findings to formulate strategies aimed at improving wheat productivity and resilience in the region, thereby contributing to food security and agricultural sustainability.

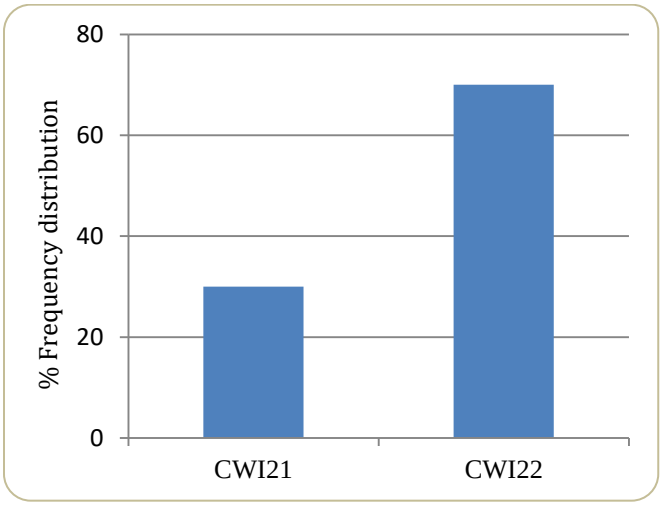


Figure 3: Frequency distribution showing presence of CWI21 and CWI22 markers in wheat genotypes.

The discovery of cell wall invertase in 75% of the varieties indicates its widespread presence and suggests that it likely plays a significant role in the function or characteristics of these cells. Understanding the role of cell wall invertase in these cells could provide valuable insights into fundamental biological mechanisms and potentially inform strategies for improving crop productivity or resilience in the face of environmental challenges. It is interesting to note that in 25% of the varieties we studied, the marker for cell wall invertase was absent. This finding raises questions about the diversity of these cells and suggests that there may be different types or subgroups within this larger category of varieties. It could also indicate that there are alternative mechanisms or pathways at play in these particular cells, which could be influencing their function or behavior differently. Further investigation into why these varieties lack the marker could provide valuable insights into the complexity of cellular processes and the factors that contribute to variation within plant populations.

## CONCLUSION

In conclusion, the study revealed a significant distribution of CWI markers, particularly CWi21 and CWi22, among wheat varieties in Sindh, Pakistan, with implications for breeding programs and agricultural policy. These markers are associated with crucial agronomic traits such as yield potential and stress tolerance. Moreover, the widespread presence of cell wall invertase in 75% of the varieties suggests its importance in cellular function, while the absence of the marker in 25% raises questions about cellular diversity and alternative mechanisms influencing cellular behavior. Further investigation could provide valuable insights into plant population variation and cellular processes.

## AUTHORS' CONTRIBUTIONS

ARM designed the study, conducted the experiments, collected data and wrote the paper; HB and MSS provided technical assistance and helped in writing the manuscript and proofread it.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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