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Molecular characterization of the genetics of abiotic stress in some wheat genotypes (*Triticum aestivum)*

BY

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SUMMARY

This study aims to evaluate the performance of four wheat parents and their crosses under stress conditions to identify superior genotypes in terms of productivity and stress tolerance, assess a range of genetic parameters, and explore the potential for enhancing wheat breeding programs by identifying promising parental lines and stress-tolerant crosses using molecular markers associated with stress tolerance.

This study was conducted at the experimental Damo Farm, Faculty of Agriculture, Fayoum University, Egypt during the growing seasons of 2019/2020, 2020/2021 and 2021/2022. Three sites with varying salinity levels control (3.56 dS/m), moderate (7.72 dS/m) and severe salinity (11.71 dS/m) were selected for experiment. The design used was a randomized complete block design (RCBD) with three replications per experiment. Four wheat genotypes derived from crosses between Sakha93, Gemmiza5 and Sids1 were evaluated under these different salinity conditions. During the first season (2019-2020) parental strains and their F_1 crosses were tested. In subsequent seasons additional F_1, BC_1 and BC_2 crosses were generated, and F_2 generation crosses were evaluated. Various agronomic, physiological, and biochemical traits were recorded including growth attributes and proline content. Key harvest traits such as plant height, number of tillers,

spike length and grain yield per plant were measured at harvest.

Data analysis was performed using Griffing's method 1, model 1 in F_1 generation to estimate general, specific combining abilities and reciprocal effect (GCA, SCA and RE) along with heterosis, potence ratio and genetic components. Genetic parameters like genetic variance, heritability and genetic advance in six populations and inbreeding depression in F_2 generation were also evaluated to understand the genetic potential of the wheat genotypes under saline stress conditions.

5.1. Combining Ability

5.1.1 Analysis of Variance and Mean Performance of Traits

Significant variation was observed for GCA, SCA and RE across studied traits in bread wheat under salt stress indicating importance of genetic components. GCA and SCA demonstrated significant roles under all stress levels with additive genetic effects predominantly influencing plant height and yield traits. GCA values were higher for plant height under low stress while SCA values were elevated for grain traits across stress conditions. The RE was found to influence traits such as spikelet per spike, proline content and biological yield underlining its importance in reducing experimental errors.

5.1.2. Mean Performance of Parental Lines and F_1 Crosses

Heading Date (HD, days): Parental lines P_1, P_2 and P_3 exhibited late heading under low stress, while P_4 flowered earliest at 70 days. Salinity conditions accelerated flowering reducing HD by 4–5 days in parental lines. Among crosses earliest HD was recorded for $P_1 \times P_3$, requiring 66.60 days under low stress. Reciprocal crosses mirrored this trend with P_3×P_1 flowering in 65.93 days under similar conditions. Salinity stress further reduced HD by 2–3 days across crosses.

Physiological Maturity (PM, days): Parental lines matured earlier under salinity stress with P4 showing shortest duration of 120.47 days under low stress. Crosses exhibited earlier PM compared to their parents with P_1×P_3 maturing fastest at 113.93 days under low stress. Under salinity crosses matured 5–7 days earlier than their parental counterparts continuing the trend of accelerated PM in reciprocal crosses.

Proline Content (µg g^{-1}): Proline content increased with salinity stress peaking in P_2 at 3.129 µg g^{-1} under severe conditions. Stable levels were observed under low stress ranging from 0.374 to 0.442 µg g^{-1} across parents. Among crosses, P_2×P_4 showed the highest proline at 3.080 µg g^{-1} under severe stress, followed by reciprocal crosses like P_4×P_2, which reached 3.137 µg g^{-1}.

Plant Height (PH, cm): PH decreased under salinity stress for all genotypes. Parental line P_3 was tallest under low stress (122.05 cm) while P_4 experienced greatest reduction dropping to 94.79 cm under severe stress. Among crosses P_2×P_4 showed shortest height under low stress (114.67 cm) and severe stress (96.27 cm) with reciprocal crosses following similar patterns.

Spike Length (SL, cm): Parental lines exhibited reduced SL under salinity stress with P_4 showing steepest decline. Crosses recorded taller SL under low stress with P_2×P_4 reaching 18.20 cm but a reduction was observed under severe stress where SL ranged from 11.60 to 14.00 cm across crosses and reciprocals.

Number of tillers (NTT): NTT decreased under stress with parental P_4 experiencing largest reduction. Among crosses $P_2 \times P_4$ recorded the highest NTT under low stress (8.00) and maintained relative performance under severe stress (7.00). Reciprocal crosses followed a similar trend.

Number of Spikelets per Spike (SS): SS decreased under salinity with P_4 showing highest reduction among parents. Cross $P_2 \times P_4$ recorded the highest SS under low stress (27.00) but stress reduced SS to approximately 23.00. Reciprocal crosses displayed comparable trends.

Number of Grains per Spike (GS): Parental P_2 exhibited the highest GS under low stress (67.71) while P_4 faced a severe reduction under salinity. Cross $P_2 \times P_4$ achieved maximum GS under low stress (76.67) but showed a reduction under severe stress (61.93). Reciprocal crosses exhibited similar reductions.

Grain Weight per Spike (GWS, g): Under low stress the highest GWS among parents was recorded for P_4 (3.43 g) while crosses $P_3 \times P_4$ and $P_1 \times P_3$ achieved the highest values (3.66 g). Severe stress caused the largest GWS reduction in P_4 (1.80 g) and cross $P_2 \times P_4$ (2.88 g). Reciprocal crosses showed similar trends under all stress levels.

100-Grain Weight (100-GW, g): P_4 exhibited the highest 100-GW (5.02 g) under low stress with a significant reduction of 2.57 g under severe stress. The cross $P_2 \times P_4$ recorded maximum value (5.52 g) under low stress decreasing to 4.65 g under severe conditions. Reciprocal crosses followed comparable patterns.

Grain Yield Per Plant (GYP, g): P_4 achieved the highest GYP (18.99 g) under low stress but experienced largest decline (10.54 g) under severe stress. Cross P_2×P_4 showed the highest GYP (29.14 g) under low stress reducing to 17.41 g under severe conditions. Reciprocal crosses exhibited a similar trend.

Biological Yield Per Plant (BYP, g): Under low stress P_2 showed the highest BYP (47.10 g) while P_4 had greatest reduction under severe stress (29.70 g). Among crosses P_2×P_4 recorded the highest BYP (68.60 g) under low stress reducing to 42.26 g under severe conditions. Reciprocal crosses followed a consistent pattern.

Straw Yield Per Plant (SYP, g): P_2 showed the highest SYP (28.36 g) under low stress with P_4 exhibiting largest decline (19.16 g) under severe conditions. Cross P_2×P_4 had the highest SYP (39.46 g) under low stress decreasing to 24.85 g under severe stress.

Harvest Index (HI, %): Under low stress P_3 exhibited the highest HI (40.95%) while P_4 showed the largest decline under severe stress (35.89%). Among crosses, P_2×P_4 recorded the highest HI (42.49%) under low stress reducing to 41.22% under severe stress. Reciprocal crosses displayed similar patterns.

5.1.3. Estimation of general (GCA), and specific combining ability (SCA) and reciprocal effects (RE).

Heading Date (HD): Parent P_1 and P_2 showed positive GCA values for HD under all stress levels making them strong combiners. Cross P_1×P_4 was best specific combiner particularly under moderate stress.

Physiological Maturity (PM): Parents P_1 and P_2 exhibited positive GCA values, especially under moderate to severe stress suggesting they are good combiners for PM. Cross $P_1 \times P_4$ was the best for PM enhancement under stress.

Proline Content: Parent P_4 had consistently positive GCA values indicating strong potential for improving proline content. Cross $P_1 \times P_3$ showed promising SCA values especially under severe stress.

Plant Height (PH): Parent P_3 exhibited positive GCA values suggesting its potential as a good general combiner for PH. Cross $P_1 \times P_4$ was the best for improving PH under stress conditions.

Spike Length (SL): Parent P_3 showed positive GCA values across all stress levels making it effective for SL improvement. Crosses $P_2 \times P_4$ and $P_3 \times P_4$ were best specific combiners under stress.

Number of tillers (NTT): Parent P_3 exhibited positive GCA values particularly under severe stress. Crosses $P_2 \times P_4$ and $P_3 \times P_4$ were identified as the best specific combiners for NTT.

Number of Spikelets per Spike (SS): Parent P_3 showed positive GCA values, making it a good general combiner for SS. Cross $P_2 \times P_4$ was the best specific combiner for SS particularly under stress.

Number of Grains per Spike (GS): Parent P_3 demonstrated positive GCA values for GS under stress. Crosses $P_2 \times P_4$ and $P_3 \times P_4$ were the best specific combiners for GS.

Grain Weight per Spike (GWS): Parent P_3 had positive GCA values, indicating its potential to improve GWS under stress. Cross $P_1 \times P_4$ was the best specific combiner for GWS.

100-Grain Weight (100-GW): Parent P_3 showed positive GCA values particularly under moderate and severe stress. Crosses $P_2 \times P_4$ and $P_3 \times P_4$ were best specific combiners for 100-GW.

Grain Yield per Plant (GYP): Parent P_3 demonstrated positive GCA values for GYP suggesting it is a good general combiner. Cross $P_2 \times P_4$ was the best specific combiner under stress conditions.

Biological Yield per Plant (BYP): Parent P_2 showed positive GCA across all stress levels especially under severe stress. Crosses $P_1 \times P_3$ and $P_2 \times P_4$ had strong positive SCA values with $P_2 \times P_4$ performing best under severe stress. Cross $P_2 \times P_3$ had negative SCA values reflecting poor performance.

Straw Yield per Plant (SYP): Parent P_1 and P_2 exhibited positive GCA values under all stress levels. Crosses $P_1 \times P_3$ and $P_2 \times P_4$ performed well under low and moderate stress. $P_2 \times P_3$ showed poor performance with negative SCA values.

Harvest Index (HI): Parent P_1 showed positive GCA especially under severe stress, while P_2 had negative GCA. Crosses $P_1 \times P_2$, $P_2 \times P_4$ and $P_3 \times P_4$ showed strong positive SCA values particularly under severe stress while $P_2 \times P_1$ and $P_3 \times P_1$ showed minimal impact.

5.1.4. Potence ratio in F_1

Potence ratio (PR) values are used to assess the dominance of traits in wheat crosses under varying levels of salinity stress. A PR exceeding ±1 indicates overdominance while values between -1 and $^{+}1$ reflect partial dominance with 0 suggesting no dominance. Under low stress crosses like P_2×P_3 show significant reductions in PR for traits such as heading date and physiological maturity with delays in development while others like P_1×P_4 exhibit stable PR values. Under moderate stress crosses like P_2×P_3 and P_3×P_2 recover significantly indicating improved adaptability. In severe stress some crosses like P_1×P_4 maintain stability while others like P_2×P_3 show continued delays. PR values for proline content increase under moderate stress indicating an enhanced stress response, but under severe stress the PR values for proline become more variable. For plant height crosses generally show negative PR values under low stress indicating a decrease compared to parental averages. However, under moderate stress some crosses recover and overdominance is observed in crosses like P_1×P_4. Spike length shows similar trends with some crosses displaying increased length under low stress but reductions under moderate and severe stress. In terms of the number of tillers crosses like P_1×P_3 show increases under low stress but a decline is seen under moderate and severe stress. The number of spikelets per spike and the number of grains per spike generally show strong performance under low stress with crosses like P_1×P_3 exhibiting overdominance. However, stress conditions lead to declines in these traits though some crosses maintain resilience. Grain weight per spike and 100-grain weight also exhibit reductions under stress with crosses like P_1×P_4 showing resilience. Grain yield per plant shows strong overdominance under low stress, but severe stress causes significant declines with some crosses like P_2×P_4 maintaining positive PR values. Biological yield per plant reveals variability under low stress with crosses like P_2×P_4 demonstrating substantial increases while under severe stress most crosses show declines. Straw yield per plant shows overdominance under low stress but a decline is observed under severe stress. Lastly the harvest index generally increases under moderate stress, with crosses like P_1×P_3 and P_3×P_1 demonstrating strong resilience under severe stress maintaining high PR values.

5.1.5. Heterosis in F_1 crosses

The heterosis data for wheat yield and its components in the F_1 generation under various salinity stress conditions reveal distinct patterns of mid-parent heterosis (MPH) and better-parent heterosis (BPH). For heading date significant negative MPH and BPH values were observed in crosses like P_1×P_3 and P_3×P_1 under low, moderate and severe salinity indicating early heading and strong adaptability. Physiological maturity data indicated positive MPH in crosses like P_1×P_4 with enhanced maturity observed under moderate salinity while crosses like P_2×P_3 showed delays. Proline content showed remarkable increases in crosses such as P_3×P_2 and P_3×P_1 under low salinity with BPH values of up to 48.83%. For plant height crosses such as P_2×P_3 and P_3×P_2 demonstrated improved growth under low salinity while others like P_1×P_3 and P_2×P_4 experienced reductions. Spike length also varied significantly with crosses such as P_4×P_3 maintaining positive MPH values under severe stress showcasing resilience. The number of tillers exhibited positive MPH values in crosses like $P_2×P_4$ under low and moderate stress while the number of spikelets per spike showed increased performance in crosses such as $P_1×P_3$ and $P_2×P_4$ under low salinity. Grain yield per plant demonstrated substantial positive MPH in crosses like $P_2×P_4$ and $P_4×P_2$ which showed resilience under all levels of salinity. In contrast, crosses like $P_3×P_2$ exhibited significant declines under severe stress reflecting the detrimental effects of salinity. Biological yield per plant showed similar patterns with crosses like $P_2×P_4$ exhibiting improved performance under stress. The crosses such as $P_2×P_4$ and $P_4×P_2$ showed significant improvements in harvest index and straw yield per plant under saline stress conditions.

5.1.6. Genetic variance components, heritability and contribution

The genetic variance components, heritability and contributions for various traits under low, moderate and severe salinity stress were analyzed. For low salinity higher additive variance was observed for traits plant height (PH) and biological yield per plant (BYP) indicating that additive genetic effects are important for these traits and can be improved through selection. Dominant variance was highest for heading date (HD), straw yield per plant (SYP) and grain spike (GS) suggesting non-additive gene actions play a role particularly benefiting cross performance. Environmental variance was relatively low, highlighting the stability of genetic factors in these conditions. The heritability estimates indicated that most traits had high broad sense heritability (above 98%) confirming dominant role of genetics. Narrow-sense heritability was also high for traits like spikelet number (SS), 100-grain weight (100-GW) and NTT suggesting they are highly responsive to selection. Crosses contributed significantly to traits grain yield per plant (GYP) and SYP indicating importance of nonadditive genetic effects.

For moderate salinity additive variance declined slightly for traits like spike length (SL) and PH but they remained responsive to selection. Dominant variance increased for traits like GS and SYP emphasizing the role of nonadditive effects. Heritability remained high with increased genetic variation for proline. Crosses again showed high contributions confirming importance of cross breeding for stress tolerance.

Under severe salinity additive variance remained high for PH and SL indicating their potential for improvement through selection even under stress. Dominant variance increased for GS and SS highlighting the importance of nonadditive effects in these traits. GCV and PCV increased under severe stress suggesting greater genetic and phenotypic variation. High heritability values indicated that most traits are governed by genetic factors. Parent contributions were prominent for PH and proline content underscoring the role of additive genetic effects in improving stress tolerance. Crosses showed dominant contributions for yield related traits reinforcing effectiveness of hybridization under severe stress. Reciprocal crosses contributed less but higher values for proline suggested a maternal effect indicating the need for breeding strategies incorporating maternal inheritance to enhance stress resilience.

5.2. Six population

5.2.1. Mean performance in six populations (P_1, P_2, F_1 , F_2 , BC_1, BC_2)

The analysis of variance for the genetic structures of the F_1 generation and its parents showed no significant differences between the data from the first season (previously analyzed using Griffing's method) and the data from the second season currently being analyzed for these six populations. Accordingly, the discussion based on the Griffing analysis from the first season is considered sufficient, and the interpretation and commentary will focus on the results of the F_2 generation and the two backcross generations (BC_1 and BC_2).

The agronomic performance of twelve F_2 generation wheat crosses was analyzed under low, moderate and severe salinity stress conditions focusing on growth and yield related traits. Heading date was delayed under stress with the earliest heading observed in $P_2×P_1$ under low stress and $P_3×P_1$ under moderate stress. Physiological maturity also showed accelerated maturation under stress particularly in $P_2×P_3$ reflecting a stress induced mechanism to complete the life cycle before stress damages the plant. Proline increased under all stress conditions with the highest accumulation observed in $P_1×P_3$ under severe stress indicating its role in stress tolerance. Plant height decreased under increasing stress with the tallest plants observed in $P_1×P_2$ under low stress and shortest under severe stress in $P_3×P_4$. Spike length and number of tillers also showed reductions under stress with the longest spikes in $P_2×P_4$ under low stress and the highest NTT in $P_2×P_4$ under low and severe stress. Spikelet number and grain spike were adversely affected by stress with reductions in grain number under moderate and severe stress particularly in P_3×P_4. Grain weight per spike and 100-grain weight decreased under severe stress, with the lowest values observed in P_3×P_1. Grain yield per plant and biological yield per plant showed significant reductions under severe stress with the highest yields in P_4×P_2 under low stress and P_2×P_4 under moderate stress. Straw yield per plant mirrored trends seen in biological yield, with the highest values in P_4×P_1 under low stress and P_2×P_4 under moderate stress. Harvest index was highest in P_1×P_3 under low stress and P_2×P_4 under moderate stress with severe stress leading to a reduction in HI across most crosses.

Variations in all study traits were observed under varying salinity stress levels across two backcross generations (BC_1 and BC_2). Early heading was recorded in P_2×P_4 under low stress while delayed heading was observed in other crosses. Physiological maturity was shortest in BC_1 for P_2×P_4 under low stress and longest for P_3×P_2. Severe stress reduced PM in BC_1 but delayed it in BC_2 with P_2×P_4 showing efficient resource use. Proline content increased progressively under higher stress with P_4×P_2 showing the highest accumulation under severe stress. Plant height showed a reduction under moderate and severe stress with P_3×P_2 exhibiting elongation under high stress. Spike length declined with increasing stress, but P_2×P_4 consistently maintained the longest spikes. Number of tillers, spikelets and grains per spike decreased under higher stress with P_2×P_4 performing better in terms of tillering and reproductive success. Grain weight per spike varied across crosses with P_2×P_4 maintaining the highest values especially under low salinity. The 100-grain weight showed minimal variation under stress, with slight reductions under severe conditions. Grain yield per plant also decreased under moderate and severe stress but P_2×P_4 demonstrated better stress tolerance and higher yields compared to other crosses. Biomass accumulation, reflected by BYP and SYP was higher under low stress with P_2×P_4 showing superior performance. Despite reductions under higher stress P_2×P_4 continued to perform better in terms of both biological and straw yield. Harvest index showed minimal variation under low stress, but severe stress led to a decrease particularly in P_3×P_2, indicating reduced reproductive success under extreme salinity conditions. These findings highlight adaptability of P_2×P_4 across various stress conditions with strong performance in grain yield and biomass accumulation under both low and high salinity stress.

5.2.2. Inbreeding depression

Inbreeding depression (ID) was observed across all traits indicating the negative effects of close breeding under various stress conditions (low, moderate and severe). Significant variations in heading date were seen with P_4×P_2 showing minimal ID under low stress indicating early heading while P_1×P_4 displayed high stability under severe stress. Physiological maturity varied with P_2×P_4 showing the least ID under low and moderate stress and P_2×P_1 demonstrating better adaptability under severe stress. Proline content was highest in P_3×P_2 under low stress indicating strong osmotic adjustment and remained robust under moderate stress with P_2×P_3 showing effective adaptation under severe stress. Plant height was best in P_2×P_3 under low stress while P_3×P_1 exhibited the highest height under severe stress. Spike length was longest in P_1×P_3 under low stress and P_3×P_4 showed minimal ID under severe stress. The number of tillers was most stable in P_2×P_4 under low stress and P_3×P_4 under moderate and severe stress. Spikelet number was highest in P_4×P_1 under low stress, while P_3×P_4 excelled under severe stress. The number of grains per spike was highest in P_4×P_1 under low stress and P_3×P_4 demonstrated resilience under severe stress. Grain weight per spike was most favorable in P_3×P_1 which showed positive ID across all stress levels. The 100-grain weight was highest in P_4×P_2 under low stress and remained stable under severe stress. Grain yield per plant was highest in P_4×P_3 under low stress, with P_3×P_1 showing the best performance under moderate and severe stress. Biological yield was highest in P_4×P_3 under low stress and in P_3×P_1 under moderate and severe stress. Straw yield was best in P_3×P_1 across all stress conditions, confirming its strong genetic potential. Harvest index was highest in P_4×P_1 under low stress and remained stable in P_4×P_2 under moderate and severe stress.

5.2.3. Scaling tests (A, B, C)

The scaling tests (A, B, and C) conducted on six populations were used to examine gene actions influencing various traits under different salinity conditions. Under low salinity P_4×P_1 showed dominant contributions while P_2×P_4 exhibited poor performance due to recessive alleles. In moderate salinity P_1×P_3 and P_2×P_1 demonstrated strong salinity tolerance, while P_2×P_3 underperformed due to recessive alleles. In severe salinity P_4×P_3 showed the best performance, with P_2×P_4 showing poor performance due to negative alleles. For physiological maturity crosses such as P_2×P_4 showed delayed PM under low salinity, while P_1×P_3 and P_2×P_1 showed better performance under moderate conditions. Under severe stress

$P_4 \times P_3$ performed best. For proline content crosses involving P_4 demonstrated better resilience under low and moderate stress conditions while P_3 crosses showed more variability. Plant height decreased in most crosses under low salinity with crosses like $P_1 \times P_2$ showing particularly low PH due to recessive alleles. In moderate and severe salinity crosses with P_4 showed positive results suggesting the beneficial role of P_4 alleles. For spike length crosses involving P_4 such as $P_4 \times P_1$ performed best under moderate and severe salinity. Similarly, crosses with P_4 alleles showed strong performance in tiller number, grain weight per spike and number of grains per spike under various stress levels. Crosses like $P_4 \times P_1$ and $P_4 \times P_2$ exhibited superior performance in grain yield per plant and biological yield per plant especially under moderate and severe salinity. Straw yield per plant was higher in crosses like $P_4 \times P_1$ and $P_4 \times P_2$ which demonstrated better tolerance across salinity conditions. The harvest index was highest in crosses involving $P_4 \times P_1$, $P_4 \times P_2$ and $P_2 \times P_1$, indicating strong resilience to salinity stress.

5.2.4. Genetic Model Parameters

The analysis of genetic model parameters showed that the performance of crosses for various traits is influenced by both additive and nonadditive genetic effects, with dominance and epistatic interactions playing a key role under stress conditions. It was observed that cross $P_1 \times P_4$ exhibited the best performance for heading date under severe stress with a high additive gene effect, indicating stress tolerance. Dominance and epistatic interactions were also significant, especially for crosses $P_4 \times P_2$ and $P_4 \times P_1$. Similarly, cross $P_2 \times P_4$ showed early physiological maturity under severe stress with strong additive effects and dominance contributions. Proline content analysis revealed that nonadditive effects predominated, especially under severe stress, with cross $P_4 \times P_1$ displaying the highest proline content under all stress levels. Dominance effects on plant height were critical across stress conditions, with cross $P_4 \times P_1$ being stable under low stress. Spike length was mainly influenced by nonadditive gene actions, with crosses $P_4 \times P_2$ performing well across stress levels. Regarding yield-related traits, crosses $P_4 \times P_1$ and $P_4 \times P_2$ exhibited superior performances for grain yield per plant and biological yield per plant, particularly under moderate and severe stress, driven by dominance effects. Straw yield per plant was highest for $P_4 \times P_1$ under low stress, while $P_4 \times P_3$ excelled under severe stress. The harvest index was significantly affected by both additive and nonadditive effects, with cross $P_1 \times P_3$ showing resilience under severe conditions.

5.2.5. Genetic components, heritability and genetic advance

Genetic components of variance, degree of dominance, heritability, genetic advance and gain from selection were evaluated across bread wheat traits under low, moderate and severe stress conditions. Under low stress environmental variance predominated for heading date (HD) and physiological maturity (PM) while additive variance was significant for plant height (PH) and number of spikelets per spike. Dominance variance was high for HD and PM suggesting substantial nonadditive effects. Overdominance was observed for HD and spike length. As stress increased to moderate levels additive variance increased for PH and biological yield per plant (BYP) and dominance variance grew for PH and number of grains per spike. Heritability was high for PH and BYP under moderate stress. Under severe stress environmental variance dominated traits like BYP and grain weight per spike. Additive variance decreased significantly and dominance variance increased for PH and BYP emphasizing nonadditive effects. Broad sense heritability remained high for BYP and harvest index, while narrow sense heritability dropped sharply for PH and PM. Genetic advance was notable for BYP and PH with selection gain highest for these traits under severe stress reflecting their importance for breeding under high-stress conditions.

5.3. Molecular study

5.3.1. DNA yield and quality

Genomic DNA was successfully extracted from different wheat genotypes using CTAB method, as confirmed by distinct DNA bands on agarose gel electrophoresis. Variations in band intensity suggested differences in DNA concentration and quality among samples. PCR amplification targeting SOS1 gene demonstrated successful amplification in most parental (P_1, P_2, P_3), and F_1 individuals, except P_4, where no band was detected, possibly due to gene absence or mutation. The inheritance of SOS1 in F_1 individuals followed mendelian patterns, combining parental alleles, while F_2 individuals showed greater banding variability, reflecting segregation and recombination.

5.3.2. Genetic relationship of wheat genotypes

Phylogenetic analyses based on SOS1 gene sequences revealed significant genetic diversity among wheat genotypes. Line G3-8 clustered closely with *Triticum turgidum* and

Triticum aestivum, indicating a shared ancestry with domesticated wheat. In contrast, Line G1-4 showed close genetic proximity to the Sakha 8 cultivar and more distant relationships with wild relatives like *Aegilops species*, suggesting domestication-related divergence.

The SOS1 gene, encoding a Na^+/H^+ antiporter critical for salt tolerance, exhibited conserved functional domains across genotypes, underscoring its importance in ionic homeostasis. Phylogenetic trees demonstrated evolutionary relationships and highlighted potential candidates for breeding programs aiming to improve salt tolerance in wheat.

Discussion emphasized that the clear PCR results, expected mendelian segregation in F_2 generations, and observed genetic diversity align with previous research on SOS1's role in salinity stress adaptation. The study proposed the use of SOS1 as a molecular marker for marker-assisted selection (MAS) in wheat improvement programs. Future research should focus on sequencing SOS1 polymorphisms and profiling gene expression under stress to better understand its contribution to salt tolerance.