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Identification of potential morpho-biochemical determinants conferring salt tolerance in wheat (*Triticum aestivum* L.) through seedling- and -reproductive stage phenotyping

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Abstract

To optimize a reproductive-stage-specific phenotyping protocol and isolate potential determinants conferring salinity tolerance in wheat, two consecutive experiments were conducted using salt-tolerant varieties (Binagom-1 and BARI Gom 25) and a sensitive variety (BARI Gom 20). In the first experiment, seedlings were grown hydroponically, and 14-day-old seedlings were subjected to two different levels of salt stress (EC=12 dS/m and 16 dS/m) for 7 days. Based on the results of tolerant and susceptible varieties, parameters such as root and shoot weight, shoot Na^+/K^+ ratio, chlorophyll content, proline content, methylglyoxal content, H_2O_2 content and lipid peroxidation content and the activities of enzymes such as ascorbate peroxidase, peroxidase, and glyoxalase I were considered as potential determinants of salt tolerance. The second experiment employed four leaf cutting treatments under both control and salinity stress conditions. Seedlings were grown in perforated pots filled with field soil, and at the heading stage, plants were subjected to salt stress (12 dS/m) after trimming as indicated. The combined analysis of control and salt stress data obtained from setup B reflected a significant decrease in yield and yield-attributing traits; however, a lesser decrease was observed in tolerant varieties. Correlation studies revealed that grain yield per spike exhibited a significant positive correlation with the number of seeds per spike, spike weight, plant height, and days to first flowering under both stress and control conditions. Additionally, different stress tolerance indices also supported the results of reproductive stage phenotyping. However, further studies will be required to tag the genes/QTLs controlling salinity tolerance in wheat at various growth phases.

Keywords

salinity screening; phenotyping; leaf cutting; seedling stage salt tolerance; reproductive stage salt tolerance; oxidative stress; stress indices

Introduction

With a global production exceeding 700 million tons and meeting 20% of the daily protein and calorie needs for 4.5 billion people, wheat (*Triticum aestivum* L.), sometimes known as the "king of cereals", is a significant cereal crop in many regions of the world (1). In Bangladesh, wheat is considered the second-largest cereal crop after rice in terms of production. It is a crucial Rabi season crop in Bangladesh, requiring 2-4 times less water compared to

rice. From 1961 to 2013, Bangladesh's annual per capita wheat consumption increased by 102%, from 8.62 to 17.47 kg (2). The national consumption of wheat in Bangladesh is about four times higher than average annual domestic production. Therefore, we need to increase wheat production to fulfil the domestic demand. In the contrary, salt stress resulted in a significant impact on wheat productivity in Bangladesh's saline-prone coastal zone. A large area (more than 1.2 million hectares) of the cultivable lands in the coastal areas remains fallow during the winter season (November–May) due to excessive salt concentration in the soil (3). These regions have exceptionally low agricultural land utilization, significantly lower than the national average for crop intensity. Therefore, we have a great opportunity to utilize this fallow area by cultivating salt tolerant wheat variety particularly during the winter season to ensure food security as well as to increase cropping intensity.

The effects of salinity arise from intricate interactions between morphological, physiological, and biochemical processes, such as seed germination, plant growth, and water and nutrient uptake. Ionic toxicity and osmotic stress pose the main challenges for plants under salt stress (4). Salinity affects almost all phases of plant growth, including germination, vegetative growth, and reproductive development. The sudden increase in reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), superoxide ($O_2^{\bullet-}$), and hydroxyl radical ($^{\bullet}OH$), which ultimately leads to oxidative stress, is one of the most significant biochemical characteristics of salt stress, in addition to ionic and osmotic stress (5). Additionally, side effects include decreased assimilate synthesis, slowed cell growth, dysfunctional membranes, altered metabolism, and an excessive amount of reactive carbonyl chemicals like methylglyoxal (MG) have been reported (5, 6). Due to their propensity to interact with proteins, lipids, and nucleic acids, MG and ROS produced in excess by plants harm cells and disturb cellular equilibrium. Eventually, plant growth disturbances, decreased fertility, and early senescence are caused by photosynthesis inhibition, damage to cellular structures, and metabolic dysfunction (7). The most detrimental effects of salinity during the reproductive stage are on the initiation of panicles, pollen fertility, spikelet formation, pollen germination, and fertilization. Significant effects have also been noted on spike weight, spike length, number of filled grains per spike, number of unfilled grains per spike, total grains per spike, total grain weight per spike, weight of 1000 seeds, and the number of spikes per plant (8, 9).

To cope with salt stress, wheat plant adopts a variety of strategies to overcome the challenges posed by increased salinity (10). A plant's ability to resist the negative effects of elevated salinity is believed to rely on its capacity to exclude Na^+ from the shoot. In wheat, this is primarily accomplished (>98%) by limiting net Na^+ uptake at the soil -root interface and net xylem loading in roots. Additionally, a plant's ability to survive salinity stress is largely determined by the K^+/Na^+ ratio, making it a valuable screening tool for plant breeders (11). Additionally, plants frequently accumulate proline in response to abiotic challenges like salinity stress. It is well known that plants generally respond to stresses, such as salt, by producing an excessive amount of MG and ROS (5, 12). Plants up-regulate the glyoxalase system, consisting of two enzymes, glyoxalase I and glyoxalase II, which convert MG to less toxic D-lactate, in response to MG stress in order to mitigate stress-related damage (5). Moreover, to counter ROS-induced damage, plants up-regulate both enzymatic (superoxide dismutase, SOD; catalase, CAT; peroxidase, POD; glutathione peroxidase, GPX; ascorbate peroxidase, APX; glutathione-*S*-transferase, GST; glutathione reductase, GR; dehydroascorbate reductase, DHAR; and monodehydroascorbate reductase, MDHAR) and non-enzymatic antioxidants such as glutathione (GSH), ascorbate (AsA), tocopherol, carotenoids, and flavonoids (4, 12). Although ROS and MG are often considered noxious compounds that hinder plant growth and development, at low concentrations they function as significant signaling molecules that, through signal transduction pathways, regulate the expression stress-responsive genes (13-15).

The previous few decades, there have been numerous attempts to develop salt-tolerant wheat cultivars, but they have only achieved sporadics success. One of the main reasons for the limited success in breeding salt-tolerant wheat varieties is the absence of precise indices for morpho-physiological and biochemical traits related to salinity stress tolerance at various growth stages, as well as the low genetic variability of the currently available wheat germplasm. Additionally, the lack of correlation between tolerance at the seedling and reproductive stages in wheat and rice suggests that these two sensitive stages are unique from one another and are controlled by different sets of gene (16, 17). As a result, finding appropriate agro-physiological and biochemical measures that can serve as screening criteria for differentiating between saline-tolerant and susceptible genotypes is imperative for the development of tolerant wheat genotypes (18). However, accurately defining the appropriate stage at which salinity stress should be applied to plants is essential to achieve precise growth stage-dependent phenotyping. Despite the gametophytic stage being the optimal time to apply salinity stress to plants, it takes a few days for salt to reach the inflorescence. Initially, salt travels to the oldest leaves and leaf sheath, then to the second oldest leaves and leaf sheath, and so on until it reaches the inflorescence. Applying the salt treatment when the plants display visible symptoms of booting would inevitably delay salt loading in the reproductive organs at the most suitable stage. This delay can be attributed to the systematic cascade manner in which toxic ions (Na^+) are transported from old leaves to younger leaves and eventually to the flag leaf and inflorescence (19). The hypothesis of this study was that cutting most of the old leaves of wheat plants before the imposition of salt stress would accelerate the transport of salt to the remaining leaves and inflorescence. However, the question arises: how many leaves should be retained so that leaf removal does not significantly affect grain yield? Additionally, mass screening for

salt tolerance directly in the field is challenging and comes with numerous limitations, such as a high degree of soil heterogeneity and others (20). Because fertilization and seed production take place during this stage, the reproductive stage is closely tied to grain yield, making salt tolerance at this period essential. The aim of the study is to standardize a reproductive-stage specific phenotypic protocol and to identify potential morpho-biochemical markers linked to salinity stress tolerance at various phases of plant growth. Additionally, we studied the suitability of different stress tolerance indices in distinguishing between tolerant and susceptible genotypes.

Materials and Methods

Experiment 1: Identification of salt tolerance determinants at the seedling stage

Experimental materials

Three wheat varieties, including two salt-tolerant ones (Binagom-1 and BARI Gom 25) and one salt-sensitive variety (BARI Gom 20; also known as Gourab), were used as plant materials. BARI Gom 20 and BARI Gom 25 were obtained from the Bangladesh Agricultural Research Institute (BARI), while Binagom-1 was obtained from the Bangladesh Institute of Nuclear Agriculture (BINA).

Experimental design and stress treatments

The three treatments used in the experiment were control (C), moderately saline stress (EC=12 dS/m) (MSS), and strongly saline stress (EC=16 dS/m) (SSS). The respective EC values of 12 and 16 dS/m were categorized as moderately and strongly saline stress levels based on the salinity classification provided by the Soil Resource Development Institute, Bangladesh. The experiment was conducted using a completely randomized design (CRD) with three replications.

Time and experimental site

The experiment was conducted under control conditions (Temp: 20±2°C, RH:80%) in the growth chamber of the Department of Genetics and Plant Breeding, Bangladesh Agricultural University, Mymensingh, during the period from September 2020 to September 2021.

Seedlings establishment and growth under hydroponic system

The wheat seeds were surface sterilized with 70% ethanol. They were then placed on wet tissue paper embedded in petri dishes and kept in an oven at 28^0C for 48 hrs to facilitate germination. Sprouted seeds were then sown in a line on Styrofoam sheet floating in trays containing normal water under controlled conditions. The tray dimensions were 32.50 cm × 28.50 cm × 13.00 cm (length, breadth and width, respectively), with a volume of 11 L. After 3 days of seedlings growth in normal tap water, the trays water was replaced with a half-strength nutrient solution (Peters® Prfoessional, Geldermalsen, Netherland), and pH was adjusted to 5.7-5.8 using NaOH and HCl. Seven-day-old seedlings were then transferred to full-strength nutrient solution (Peters solution) and continued for 14 days. The nutrient solution was replaced every 7 days. All procedures were conducted under controlled conditions at 20±2°C with a 16/8-hrs light/dark cycle.

Preparation of saline solution and application of salt stress treatment

The desired salinity levels were achieved by dissolving crude salt collected from the seashore until the treatment levels reached moderately saline stress (MSS) and strongly saline stress (SSS), as determined by checking the EC meter. The control group was maintained using nutrient solution only. After 14 days of seedlings growth, two groups of seedlings were subjected to two different levels of salt stress (12 dS/m and 16 dS/m) in the Peters solution for 7 days (Fig. 1). The control seedlings were grown solely in the nutrient solution.

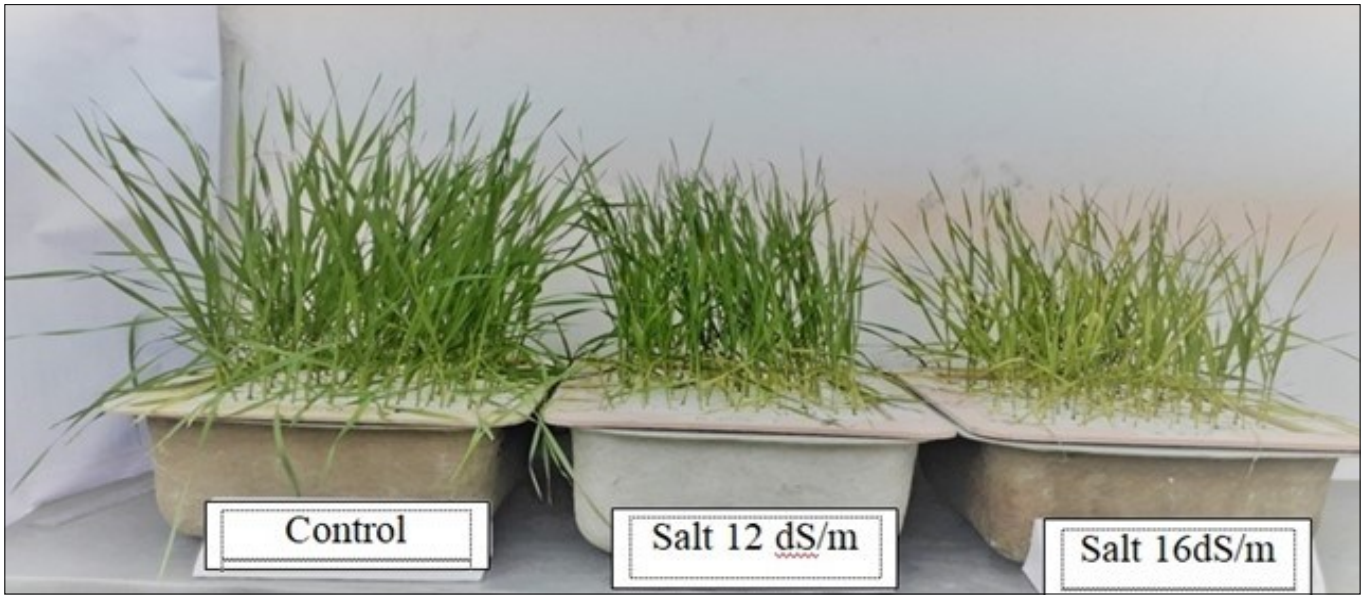

Fig. 1. Hydroponic culture of wheat seedlings grown under control and salt-stressed (12 dS/m and 16 dS/m) conditions.

Data on morphological and biochemical traits

After 7 days of salt stress treatments, various morphological traits (shoot length (SL), root length (RL), shoot fresh weight (SFW), root fresh weight (RFW), shoot dry weight

(SDW), root dry weight (RDW) were recorded from 10 randomly selected seedlings per replications. Biochemical traits such as chlorophyll content were measured according to Lichtenthaler *et al.* (21); MG level according to Rohman *et al.* (22); and Na^+ and K^+ content according to Brown and Lilleland (23). Proline, H_2O_2, MDA, and protein content were measured following standard methods as described by Islam *et al.* (24).

Enzyme extraction and activity assay

According to Hossain *et al.* (12), the enzyme was extracted from the leaf sample, and protein concentration was assessed using Bradford's (1976) techniques. Following the procedures outlined by Hossain *et al.* (12), the activities of ascorbate peroxidase (APX), glutathione S-transferase (GST), and glyoxalase-I (Gly-I) were assessed, while peroxidase (POD) activity was determined according to Hemeda and Klein (25).

Experiment 2: Standardization of reproductive-stage-specific phenotyping protocol

Experimental materials and design of the experiment

Please see the section of experiment 1.

Time and experimental site

In the potyard of BINA Mymensingh-2202, research on the effects of salt stress during the reproductive stage was conducted from the middle of November 2019 to the first week of March 2020.

Preparation of perforated pot soil

Perforated pot soil was prepared for the establishment of seedlings. The soil was spaded through with a spade, and large clods were broken into smaller pieces by hand. Cow dung and fertilizers (Urea, TSP, and MP) were mixed with the soil to provide proper nutrition. The soil used was collected from the experimental field laboratory of the Department of Genetics and Plant Breeding. It had a pH ranging from 6.5–6.7, and a sandy loam texture. A piece of thin white cotton cloth was placed on the inner side of the perforated pots. Then pots were then filled with the prepared soil and placed in the trays. Each pot contained 2.17 kg of soil.

Fertilizer application

The recommended doses of fertilizer (TSP and MP) were applied to the soil during pot soil preparation. Urea was applied in two installments; half of the portion was used during soil preparation, and the remaining half was applied after thirty days of transplanting.

Seed germination, seedling growth and establishment

The selected three varieties were germinated in petri dishes at room temperature. Pre-germinated seeds were then directly sown in perforated pots filled with field soil to facilitate better establishment and growth at the experimental site of BINA, Mymensingh-2202.

Leaf cutting and growth of plants under control and salt stress

Just before the emergence of panicles from the flag leaf (heading stage), the leaf cutting experimental setup was conducted following the process outlined by Ahmadizadeh *et al.* (19):

Setup A: No leaf cutting

Setup B: Remaining top three leaves

Setup C: Remaining flag leaf and penultimate leaf

Setup D: Only the remaining flag leaf

Leaf cutting was performed on two different sets of seedlings for each genotype. One group of plants was grown under control conditions (normal tap water), while another group of plants was subjected to salt stress (EC 12 dS/m). Every 7 days, the tray's water was replaced with normal tap water, and new salt-containing water was added, with the EC adjusted regularly using an EC meter. This process continued for 18 days. After the stress treatments, seedlings were grown under normal conditions and harvested according to their maturity.

Data collection on yield and yield contributing traits

Data on days to first flowering (DFF), days to maturity (DM), plant height (PH), spike length (SL), spike weight (SW), number of seeds per spike (NSS), 100-seed weight (100-SW), and grain yield per spike (GY) were recorded from ten randomly chosen plants.

Calculation of stress tolerance indices

Stress tolerance indices such as MP (Mean Productivity), GMP (Geometric Mean Productivity), SSI (Stress Susceptibility Index), TOL (Tolerance Index), STI (Salt Tolerance index) and YSI (Yield Stability Index) were estimated based on the data on grain yield per spike using the following equations: $GMP = \sqrt{(Yp \times Ys)}$; $MP = (Yp + Ys)/2$; $SSI = (1 - (Ys/Yp))/(1 - (\bar{Y}s/\bar{Y}p))$; $STI = (Yp \times Ys)/(\bar{Y}p)^2$; $TOL = Yp - Ys$; and $YSI = Ys/Yp$. These equations were derived from the research article published by Rahman *et al.* (26).

In these equations, Ys and Yp indicate the grain yield per spike of a given genotype under stress and normal condition, respectively. The average yield across all genotypes under normal and stressful conditions is denoted by $\bar{Y}a$ and $\bar{Y}_S$, respectively.

Statistical analysis

The statistical software program Minitab 18 (Minitab Inc. State College, Pennsylvania) was used for data analysis. Following the CRD design, a two-way analysis of variance was conducted using a mixed model with two fixed factors and random repeats. The Tukey multiple comparison test was employed to determine whether there were significant differences in treatment means at the $p < 0.05$ level.

Results and Discussion

Identification of salt tolerance determinants at the seedling stage

The results of the analysis of variance indicated that the genotypes and treatments differed significantly ($p \leq 0.001$) for all traits *viz.*, SL, RL, SFW, RFW, SDW, RDW, RL and SL ratio (R/S), shoot Na^+/K^+, and root Na^+/K^+. In G × T interaction, SL, SDW, RDW, shoot Na^+/K^+ and root Na^+/K^+ were also

found to be significant ($p \leq 0.001$). The mean performance of the varieties for different growth-attributing traits under two different levels of salt stress is presented in Table 1. In response to moderate or strongly saline stress, we observed a significant decrease in SL (7.3 and 11.11% in Binagom-1; 4.93 and 6.83% in BARI Gom 25; 6.77 and 15.83% in BARI Gom 20), SFW (16.25 and 32.50% in Binagom-1; 27.27 and 31.64% in BARI Gom 25; 33.33 and 58.85% in BARI Gom 20), RFW (17.39 and 28.26% in Binagom-1; 27.27 and 40.91% in BARI Gom 25; 29.23 and 36.58% in BARI Gom 20), SDW (8.74 and 21.36% in Binagom-1; 7.86 and 17.97% BARI Gom 25; 13.98 and 38.71% in BARI Gom 20), and RDW(9.75 and 19.51% in Binagom-1; 13.51 and 29.73% in BARI Gom 25; 14.29 and 51.43% in BARI Gom 20) compared to the control (Table 1 and Fig. 2). Similar to our results, a decrease in SL, SFW, RFW, SDW, RDW in response to salinity was also reported by others (27, 28). The decrease in the above traits in response to salinity is a result of the osmotic impact and the accumulation of high concentrations of Na^+ and Cl^-. Consequently, the availability of assimilates to growing tissues and organs decreases. A decrease in cell division in response to salt stress has also been reported (29). In contrast, a significant increase in RL (15.23% in Binagom-1; 18.89 and 50.77% in BARI Gom 25; 6.70 and 16.67% in BARI Gom 20), R/S (9.09 and 36.36% in Binagom-1; 23.81 and 59.52% in BARI Gom 25 and 16 and 34% in BARI Gom 20), shoot Na^+/K^+ (1.93- and 2.46-fold in Binagom-1; 1.24- and 2.23-fold in BARI Gom 25; 1.49- and 3.45-fold in BARI Gom 20), and root Na^+/K^+ (2.26- and 3.78-fold in Binagom-1; 2.90 - and 5.51-fold in BARI Gom 25; 2.75- and 4.85-fold in BARI Gom 20) was observed in response to 12 dS/m and 16dS/m salinity levels compared to the control (Table 1). These results were also supported by other researchers (10, 30). Numerous studies have concluded that the main mechanism preventing Na^+ accumulation in leaves involves the coordinated action of transporters mediating Na^+ unloading from the root and xylem, thereby minimizing the transfer and accumulation of physiologically toxic Na^+ in shoots, and eventually in photosynthetic tissues, mediated by the high-affinity K^+ transporter (HKT) proteins. The control of Na^+ transport and Na^+ ion compartmentalization

Table 1: Mean performances of three wheat genotypes for different morphological and biochemical traits related to growth and development under control and salt conditions at the seedling stage.

Variety	Treatment	SL (cm)	RL (cm)	SFW (g)	RFW (g)	SDW (g)	RDW (g)	RL/SL Ratio	Shoot Na^+/K^+	Root Na^+/K^+
	Control	33.56 a	18.44 b-e	0.80 a	0.46 a	0.103 a	0.041 a	0.55 cd	0.70 h	0.58 gh
BN1	12 dS/m	31.11 b	18.78 b-d	0.67 b	0.38 c	0.094 ab	0.037 b	0.60 bc	1.35 d	1.31 f
	16 dS/m	29.83 c	21.25 a	0.54 c	0.33 d	0.081 c-e	0.033 cd	0.71 a	1.72 c	2.19 c
	Control	32.63 a	13.55 f	0.79 a	0.44 ab	0.089 bc	0.037 b	0.42 e	0.99 g	0.49 h
BR25	12 dS/m	31.02 bc	16.11 e	0.64 b	0.32 d	0.082 cd	0.032 de	0.52 d	1.23 e	1.42 e
	16 dS/m	30.40 bc	20.43 ab	0.54 c	0.26 e	0.073 e	0.026 f	0.67 ab	2.21 b	2.70 b
	Control	32.66 a	16.44 de	0.81 a	0.41 bc	0.093 b	0.035 bc	0.50 d	0.71 h	0.60 g
BR20	12 dS/m	30.45 bc	17.59 c-e	0.54 c	0.29 de	0.080 de	0.030 e	0.58 cd	1.06 f	1.65 d
	16 dS/m	27.49 d	19.18 a-c	0.39 d	0.26 e	0.057 f	0.017 g	0.67 ab	2.45 a	2.91 a

Note: Different letters in the same column are significant at 5% level of probability following Tukey's method. Here, **BN 1** = Binagom-1, **BR 25** = BARI Gom 25, **BR 20** = BARI Gom 20, **SL** = Shoot Length, **RL** = Root Length, **FSW** = Shoot Fresh weight, **FRW** = Root Fresh weight, **DSW** = Shoot Dry weight, **DRW** = Root Dry weight, **R/S ratio**= Root length and Shoot length ratio.

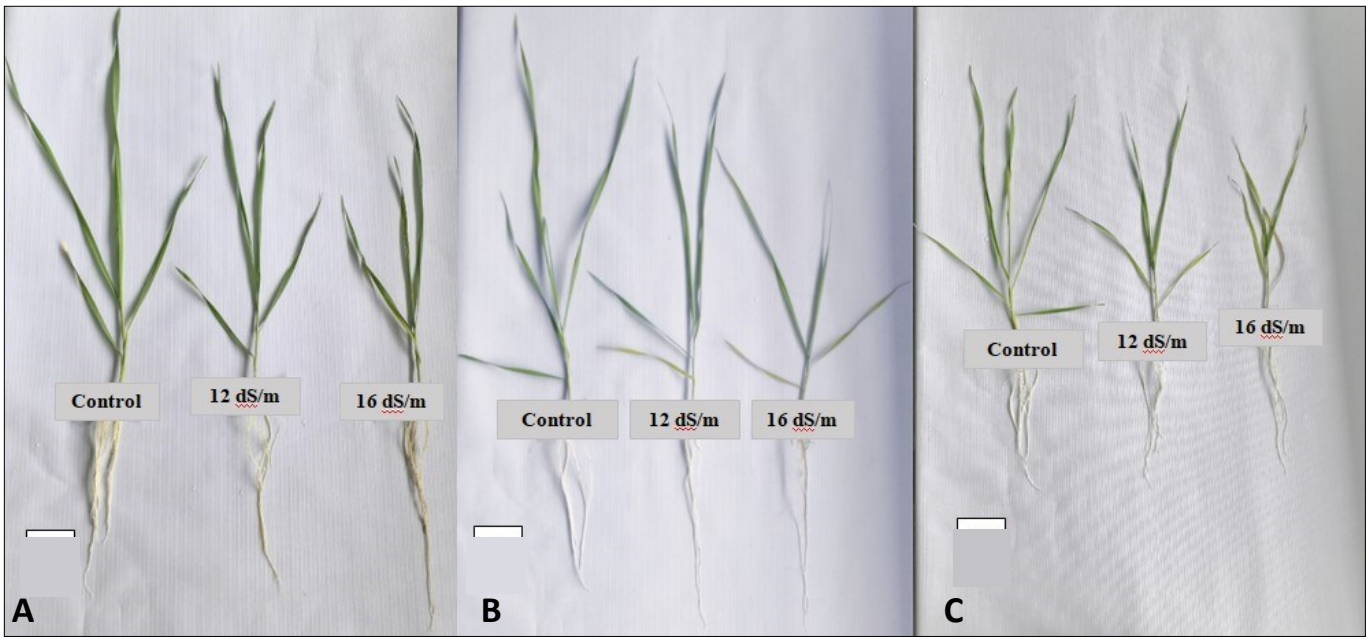

Fig. 2. Phenological apperance of seedlings grown under different levels (12 dS/m and 16 dS/m) of salt stress (**A**) Binagom -1,(**B**) BARI Gom 25 and (**C**) BRRI Gom 20.

is carried out by ion transporters like HKTs and NHXs (31). According to recent research, the ion transporter OsHKT1;5 controls the Na^+/K^+ channel by excluding Na^+ from xylem sap into xylem parenchyma cells, maintaining a low Na^+/K^+ ratio under soil salinity. It is possible that the up-regulation of salt exclusion-related genes *OsHKT1;5* and *OsNHX1* and the down-regulation of *OsHKT2;1* and *OsHKT2;2* genes led to the improved growth and yield performance observed in rice genotypes containing *SKC1* and *qSt1b* QTLs (32). Based on photosynthetic capacity, physiological parameters, and yield characteristics, it was found that the expression of these genes restricted Na^+/K^+ absorption and translocation, resulting in decreased Na toxicity. Salt-tolerant wheat varieties maintain low Na^+/K^+ ratios in shoots due to their ability to maintain ionic homeostasis, specifically low sodium-potassium ratio, through mechanism such as sodium exclusion, Na^+ compartmentation in vacuoles, and partitioning of surplus Na^+ in older plant sections.

Plenty of recent studies have also shown that plants up-regulate both enzymatic and non-enzymatic defense systems for efficient ROS scavenging and ROS-mediated stress signaling (14). The mean performance of the results of different biochemical traits, including ROS-detoxifying enzymatic activities, is presented in Table 2. Imposition of various levels of salt stress resulted in a decrease in total chlorophyll content (7.61 and 13.92% in Binagom-1, 6.15 and 16.46% in BARI Gom 25 and 19.68 and 37.08% in BARI Gom 20 under 12 dS/m and 16 dS/m salinity levels, respectively, compared to the control) (Table 2). Similar to our results, a decrease in total chlorophyll content in response to salt stress has also been reported by others (33). The decrease in chlorophyll content in response to salt stress is attributed to the inhibitory effect of salt on chlorophyll biosynthesis or the acceleration of chlorophyll degradation. Chookhampaeng (34) stated that damage to chlorophyll content is enhanced through excessive accumulation of ROS, while Zhang *et al.* (35) reported that salt stress induces swelling of chloroplast thylakoids and causes destruction of the chloroplast envelope, leading to chlorophyll reduction under salt stress. A continual increase in proline content was observed in all the varieties studied (2.5- and 7.9-fold increase in Binagom-1; 3.3- and 7.4-fold in BARI Gom 25 and 3.0- and 3.7-fold in BARI Gom 20 in response to 12 dS/m and 16 dS/m salinity levels compared to the control) (Table 2). Similarly, an increase in methylglyoxal (MG) (1.35- and 2.43-fold in Binagom-1; 1.63- and 2.35-fold in BARI Gom 25; 2.47- and 3.43-fold in BARI Gom 20 in response to 12 dS/m and 16 dS/m salinity levels compared to the control), H_2O_2 (1.5- and 1.84-fold in Binagom-1; 1.57- and 1.78-fold in BARI Gom 25; 1.57- and 2.06-fold in BARI Gom 20 in response to 12 dS/m and 16 dS/m salinity levels compared to the control), and MDA (1.08- and 1.35-fold in Binagom-1; 1.16- and 1.46-fold in BARI Gom 25; 1.55 - and 2.13-fold in BARI Gom 20 in response to 12 dS/m and 16 dS/m salinity levels compared to the control) was observed in all the varieties studied (Table 2).

Consistent with several earlier studies, proline content significantly increased in all genotypes under salinity conditions, likely resulting from new synthesis or the breakdown of proline-rich proteins during stress. This suggests that proline accumulation may serve an adaptive function related to survival rather than sustained growth, as it only occurred when growth inhibition was already severe. The highest induction was observed in the salinity-tolerant genotype Binagom-1, a result corroborated by Yassin *et al*. (36). In contrast to proline, higher accumulation of methylglyoxal (MG) exerts a negative impact on crop growth and yield. Elevated levels of MG are toxic to the cell, inhibiting cell proliferation, increasing protein degradation, and leading to the inactivation of the antioxidant defense system. The induction of MG due to salinity stress has also been reported by other researchers; however, a less pronounced decrease was recorded in the tolerant genotype (37). Similar to methylglyoxal (MG), hydrogen peroxide (H_2O_2) content also increased in all genotypes under salinity conditions. The induction of H_2O_2 was highest in the sensitive genotype BARI Gom 20 compared to the tolerant genotype Binagom-1. H_2O_2 is produced under various stress conditions such as salinity, drought, UV radia-

Table 2: Mean performances of three wheat genotypes for different biochemical and enzymatic activity traits grown under control and salt stress conditions at the seedling stage.

Variety	Treatment	Total chlorophyll (mg/mL)	Proline (µg/ g fresh sample)	MG (µmole/ g fresh weight)	H_2O_2 (µmole/g fresh weight)	MDA (nmole/g fresh weight)	APX (µmole/ min/mg protein)	POD (µmole/ min/mg protein)	GST (n mole / min /mg protein)	Gly I (µmole/ min/mg protein)
	Control	65.15 a	2.53 f	19.55 g	22.51 f	21.08 ef	0.300 a	1.03 c	0.130 bc	0.383 a
BN 1	12 dS/m	60.19 bc	6.28 e	26.41 f	33.66 e	22.79 de	0.207 c	2.21 b	0.158 a	0.291 c
	16 dS/m	56.12 cd	20.19 a	47.59 d	41.40 bc	28.46 c	0.267 b	3.18 a	0.074 d	0.219 d
	Control	64.23 ab	2.40 f	25.11 f	24.08 f	21.71 ef	0.140 d	1.02 c	0.107 c	0.232 d
BR 25	12 dS/m	60.28 a-c	10.06 cd	40.90 e	37.88 d	25.11 d	0.270 b	2.27 b	0.130 bc	0.246 d
	16 dS/m	53.66 de	17.78 b	59.01 b	42.88 b	31.66 b	0.143 d	3.13 a	0.070 d	0.140 f
	Control	62.39 ab	3.03 f	21.00 g	24.92 f	18.70 f	0.127 de	1.05 c	0.110 c	0.242 d
BR 20	12 dS/m	50.11 e	9.07 d	51.84 c	39.03 cd	29.02 bc	0.113 e	0.93 d	0.143 ab	0.183 e
	16 dS/m	39.25 f	11.35 c	71.96 a	51.23 a	39.97 a	0.098 b	1.04 c	0.063 d	0.324 b

Note: Different letters in the same column are significant at 5% level of probability following Tukey's method. Where **BN 1** = Binagom-1, **BR 25** = BARI Gom 25, **BR 20** = BARI Gom 20, **MG** = Methylglyoxal, **H_2O_2** = Hydrogen peroxide, **MDA** = Malondialdehyde, **APX** = Ascorbate peroxidase, **POD** = Peroxidase, **GST** = Glutathione-S-transferase, **Gly-I** = Glyoxalase-I.

tion, cold stress, and heat stress. Consistent with our findings, an increase in H_2O_2 levels in response to salt stress has been reported by others, although a moderate increase was also noted in tolerant genotypes (38). Hydrogen peroxide is the only ROS that can diffuse across aquaporins in membranes and over longer distances within the cell, and it is relatively stable compared to other ROS. It acts as a signaling molecule under optimum conditions, but it becomes destructive when accumulated excessively in plant cells. The increase in H_2O_2 content in response to salt stress has been reported in other studies as well (5, 39).

Lipid peroxidation, measured in terms of malondialdehyde (MDA), significantly increased in all genotypes due to salt stress imposition, with the highest increase recorded in the sensitive variety BARI Gom 20. Higher MDA content in plant cells indicates salinity sensitivity, whereas lower MDA concentration suggests resistance to oxidative stress. Previous studies (40) have indicated that increased MDA generation is associated with oxidative damage to plant cell membranes. Moreover, a lower concentration of MDA in plant cells typically corresponds to an increase in the activity of antioxidant enzymes in plant tissues, aiding the plant in surviving stressful conditions.

Ascorbate peroxidase (APX) is among the most widely distributed antioxidant enzymes in plant cells. APX plays a crucial role in regulating intracellular levels of reactive oxygen species (ROS) and is responsive to redox signals and hydrogen peroxide (H_2O_2). H_2O_2 generated in the cytosol, apoplast, or released from organelles is scavenged or detoxified by APX. In this study, we observed a gradual decrease in APX activity, with a particularly sharp reduction noted in the susceptible variety BARI Gom 20 (11.02% and 22.83% decrease in response to 12 dS/m and 16 dS/m salinity levels compared to the control). Similar decreases in APX activity have been reported in wheat, corroborating our findings (41). In contrast to APX, a gradual increase in POD activity was observed among the studied varieties, with greater induction found in the tolerant varieties Binagom-1 and BARI Gom 25 (2.1- and 3.08-fold increase in Binagom-1, and 2.2- and 2.98-fold increase in BARI Gom 25 in response to 12 dS/m and 16 dS/m salinity levels compared to the control). The induction of POD activity due to salinity has been reported by other researchers. Glutathione S-transferase (GST) has been implicated in the response to salinity in several studies, and GST-overexpressing plants demonstrate higher tolerance to salt stress (42). In the present study, the highest increase in GST activity was found under moderate salt stress in the tolerant variety. However, the activity showed a decreasing trend under strong salinity stress. Glyoxalase-I (Gly-I) activity was found to decrease with increasing salinity levels; however, it showed a sharp increase (33.88% compared to the control) under 16 dS/m salinity.

Importantly, high constitutive activities of these enzymes were found in the tolerant genotypes. Glyoxalase -I gradually decreased in the tolerant genotype due to Gly-I dependent detoxification, which is the major pathway for MG catabolism. Therefore, the tolerant genotype showed lower levels of Gly-I. This result was also supported by Hossain *et al.* (43). Based on the results of the hydroponic study, Binagom-1 and BARI Gom 25 could be considered as the most tolerant varieties, as they showed less reduction in shoot and root weight, a lower increase in the Na^+/K^+ ratio, limited increase in MG levels, and low reduction of chlorophyll content. They also exhibited higher accumulation of proline and constitutively higher activities of APX, POD, GST, and Gly-I, whereas BARI Gom 20 was classified as a sensitive variety. The phenological appearance of the seedlings in response to salt stress (Fig. 2) also reflected the morphological and biochemical alterations, highlighting the inherent capacity of salt stress tolerance in the tolerant genotypes.

Standardization of reproductive-stage-specific phenotyping protocol

Effect of leaf cutting treatments on wheat yields at the reproductive stage

Leaf cutting in rice at the late booting stage efficiently directs salt to the reproductive organs and helps in discriminating tolerant genotypes from susceptible ones (44). Therefore, to determine the degree of leaf pruning that does significantly affect wheat yield under control conditions, we conducted a leaf pruning experiment with four different treatment combinations during the reproductive phases of growth, specifically at the heading stage when the young panicle is about to emerge from the flag leaf, as indicated in the materials and methods section. The results for DF, DM, PH, SL and 100-SW showed little change due to different degrees of trimming. This is expected, as we applied the treatments just before the panicle emergence (Table 3). However, a sharp reduction was observed for SW (39.62% in BARI Gom 25 and 37.25% in Binagom-1 between the control and flag leaf treatment), NSS (24.69% in Binagom-1 and 30.40% in BAR Gom 25 in between the treatment of control and flag leaf), and YP (18.012% in BARI Gom 20, 41.35% in BARI Gom 25 and 36.07% in Binagom -1 in between the treatment of control and flag leaf). Setup A (untrimmed plants) had the highest YP, while setup D (only flag leaf remains) had the lowest YP in all the varieties studied, with significant variability observed among the treatments and varieties (Table 3). To gain a clearer understanding, we conducted a combined analysis of the data across treatments to determine the extent of leaf pruning that did not significantly differ from the control treatment (Table 4). Our findings revealed that maintaining just three leaves on the plants did not result in significant differences compared to the control (un-trimmed) plants in terms of yield. Therefore, subjecting plants to salt stress after retaining only three leaves is suitable for distinguishing between tolerant and susceptible genotypes at the reproductive stages of growth. While the flag leaf contributes 45-60% of grain yield in rice (45), it is also known that the penultimate leaf and the third upper leaf contribute to growth and grain yield. Hence, we opted to retain the top three leaves (flag leaf and top two leaves) for security reasons, aiming to minimize sink size to store excess Na^+ during its uptake. Hence, if salt stress is imposed on plants with trimmed leaves, any significant differences

observed can be attributed solely to the salt-stress itself and its duration, rather than to the leaf pruning. The results also suggest that leaf pruning may be employed to grow plants with only the top three leaves without significantly affecting yield, as such plants behave similarly to untrimmed plants. However, since the objective is to induce the impact of salt stress on the reproductive organs of the plants as quickly as possible in order to differentiate between tolerant and sensitive wheat plants at the reproductive stage, it is more appropriate to reduce the sink size for toxic salt accumulation by leaving only the top three leaves. Ahmadizadeh *et al.* (19) reported that trimming all leaves except the two leaves (flag leaf and penultimate leaf) at the reproductive stage did not significantly affect yield and yield attributing traits in rice. Therefore, they suggested maintaining at least two leaves for reproductive-stage phenotyping in rice.

BARI Gom 25 and BARI Gom 20 compared to control), SW (23.31, 25.72, 38.88%, respectively, in Binagom-1, BARI Gom 25 and BARI Gom 20 compared to control), 100-SW (22.29, 48.11, 55.22%, respectively, in Binagom-1, BARI Gom 25 and BARI Gom 20 compared to control) and GY (36.36, 53.29, 56.33%, respectively, in Binagom-1, BARI Gom 25 and BARI Gom 20 as compared to control). Importantly, a greater decrease was found in BARI Gom 20, whereas a less decrease was observed in tolerant varieties (Binagom-1 and BARI Gom 25). Salt stress inhibited normal growth and development and accelerated flowering and maturation. We observed little change in PH in response to salt stress in Binagom-1 and BARI Gom 25; however, a significant decrease was found in the salt-sensitive variety BARI Gom 20. The imposition of salt stress also resulted in a significant decrease in PH, SL, SW, NSS and 100-SW and YP, with a greater decrease observed in the salt sensitive

Table 3: Mean performances of three wheat genotypes based on different yield and yield related traits under different levels of leaf pruning.

Variety	Treatment	DFF	DM	PH (cm)	SL (cm)	SW (g)	NSS	100-SW (g)	GY (g)
BN 1	Control	60.00 a	101.33 bc	66.60 a	9.77 b-e	3.06 a	55.33 a	4.21 ab	2.19 a
	3 leaves	59.00 b	104.0 a	66.79 a	9.31 de	2.37 a-c	43.33 b	4.10 ab	1.87 a-c
	2 leaves	59.33 b	102.67 ab	65.69 ab	9.20 e	2.22 a-c	42.67 b	4.07 ab	1.64 cd
	Flag leaf	59.00 b	101.00 b-d	65.75 ab	8.79 e	1.92 bc	41.67 b	3.31 b	1.40 de
BG 25	Control	57.00 c	99.33 d-f	63.17 b-c	11.27 a	2.7 ab	41.67 b	5.14 a	2.08 ab
	3 leaves	55.00 d	98.00 f	63.24 b-d	10.93 ab	2.41 a-c	36.67 c	5.03 a	1.82 bc
	2 leaves	55.00 d	100.00 c-e	63.52 b-d	10.88 a-c	2.20 bc	30.33 ef	5.03 a	1.56 cd
	Flag leaf	55.00 d	99.67 c-f	60.69 de	10.50 a-d	1.63 c	29.00 f	4.27 ab	1.22 e
BG 20	Control	53.00 e	98.67 ef	64.03 a-c	9.63 c-e	2.02 bc	34.67 cd	4.54 ab	1. 75 c
	3 leaves	53.00 e	102.00 b	64.75 a-c	9.29 de	1.98 bc	33.67 c-e	4.32 ab	1.42 de
	2 leaves	53.00 e	102.00 b	61.95 c-e	9.11 e	1.94 bc	32.00 d-f	4.28 ab	1.41 de
	Flag leaf	53.00 e	99.00 ef	60.04 e	8.79 e	1.92 bc	31.33 d-f	4.27 ab	1.22 e

Note: Different letters in the same column are significant at 5% level of probability following Tukey's method. Where, **DFF** = Days to first flowering, **DM** = Days to maturity, **PH** = Plant height, **SL** = Spike length, **SW** = Spike weight, **NSS** = Number of seeds per spike, **100-SW** = 100-seed weight, **GY** = Grain yield per spike, **BN 1** = Binagom-1, **BG 25** = BARI Gom 25, **BG 20** = BARI Gom 20 .

Table 4: Yield per spike as affected by leaf pruning treatments.

Treatment	Mean yield/spike (g)
Control (no leaf cutting)	1.92 a
3 leaves	1.7 ab
2 leaves	1.54 bc
Flag leaf	1.28 c

Effect of salt stress on yield and yield attributing traits at the reproductive phases of growth

To evaluate the differences in yield and yield attributing traits under salt stress to assess the suitability of leaf pruning in distinguishing tolerant and susceptible genotypes, we conducted an experiment under control and salt stress conditions with varying degrees of leaf pruning (Fig. 3). We performed a combined analysis of the data obtained from setup B. The analysis of variance for all characters (*viz.* DFF, DM, PH, SL, PW, NSS, and YP) showed highly significant ($p \leq 0.001$) variation among the genotypes due to treatments. The mean values of the experiment are presented in Table 5. Salt stress resulted in a significant decrease in DM (5.8, 9.18, 10.13%, respectively, in Binagom-1,

variety. Similar to our results, a decrease in yield components, such as spikes per plant, SL, SW, filled spikelet per plant, total spikelet per plant, grain weight per plant, were also reported in wheat (46, 47). However, a greater decrease in these traits was observed in susceptible wheat genotypes (30). Salinity-induced yield reduction in wheat at the reproductive phase is mainly due to Na^+ and ROS toxicity-induced floret abortion, pollen sterility, decreased photo-assimilates, and reduced partitioning of the plant's resources toward grains (48). This may be attributed to changes in gene expression induced by salt stress during the pre-anthesis and grain filling stages, such as the hindrance of fructan build-up and carbohydrate remobilization to grains (49). However, salt-tolerant genotypes exhibited the most tillers while reducing spikelet floret abortion and showed superiority in moving photoassimilates from leaves to grains (30). According to several studies, salt stress causes early leaf senescence, reduces panicle formation, leads to excessive accumulation of Na^+ and Cl^- in floral parts, disrupts pollen viability and stigma receptivity, decrease assimilate production, and limits nutrient and carbohydrate translocation to the panicles. In addition to lowering photosynthetic pigment levels, salt stress

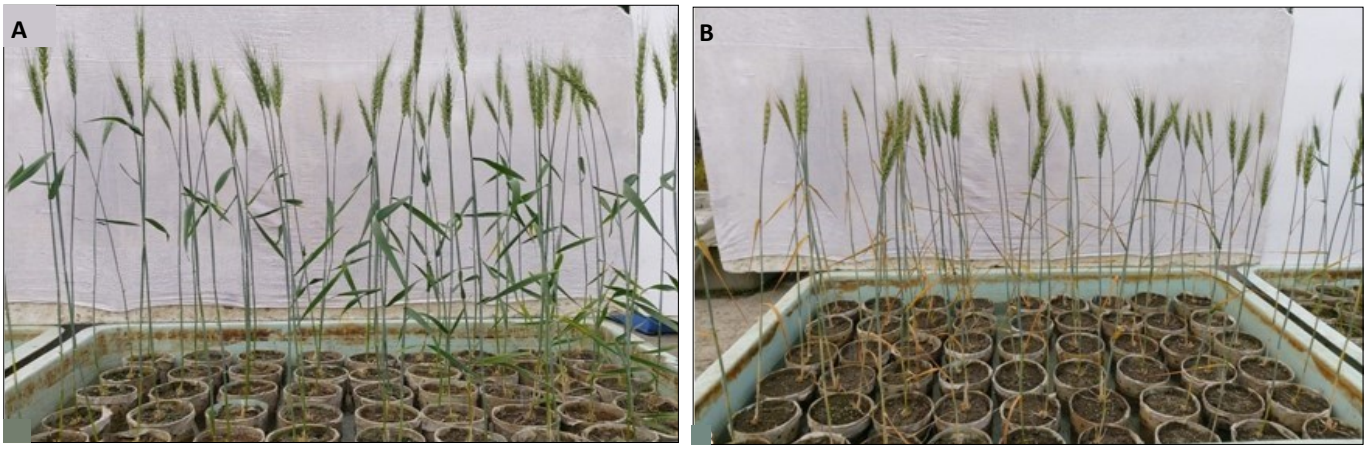

Fig. 3. Phenological appearance of plants grown under control (**A**) and salt-stressed (**B**) condition after trimming of leaves at various combinations.

Table 5: Mean performances of three wheat genotypes based on different traits related to yield grown under control and salt (12dS/m) stress conditions at the reproductive stage maintaining three leaves in the plants.

Variety	Treatment	DFF	DM	PH (cm)	PL (cm)	SW (g)	NSS	100-SW (g)	GY (g)
BN 1	Control	59.00 a	104.00 a	66.78 a	9.31 b	2.36 a	43.33 ab	4.09 a	1.87 a
	Salt	57.33 b	98.00 c	68.53 a	9.41 b	1.81 b	40.15 a	2.98 b	1.19 bc
BR 25	Control	55.00 c	98.00 c	63.24 bc	10.93 a	2.41 a	36.66 bc	5.03 a	1.82 a
	Salt	53.00 d	89.00 e	63.11 bc	11.38 a	1.79 b	32.33 c	2.61 b	0.85 cd
BR 20	Control	53.00 d	102.00 b	64.74 b	9.28 b	1.98 ab	33.66 c	4.31 a	1.42 ab
	Salt	47.00 e	91.667 d	62.45 c	9.36 b	1.21 c	34.66 c	1.93 b	0.62 d

Note: Different letters in the same column are significant at 5% level of probability following Tukey's method. **BN 1** = Binagom-1, **BR25** = BARI Gom 25, **BR20** = BARI Gom 20, **DFF** = Days to first flowering, **DM** = Days to maturity, **PH** = Plant height, **SL** = Spike length, **SW** = Spike weight, **NSS** = Number of seeds per spike, **100-SW** = 100-seed weight, **GY** = Grain yield per spike.

affects osmolyte accumulation, plant-water interactions, membrane integrity, and K^+ uptake, resulting in reduced yield and yield characteristics (50).

Association among the characters under control and stress condition at the reproductive stage

Grain yield is a complex attribute resulting from multiplicative interactions between various contributing characteristics. The effectiveness of the selection process in a breeding program depends on understanding how these characteristics interact. In the current investigation, DFF showed strong positive correlations with DM (0.446*, 0.639***), PH (0.667***, 0.644***), SW (0.484**, 0.740***), NSS (0.831***, 0.657***), and GY (0.543***, 0.797***) under both control and salt stress conditions, respectively (Table 6). Under salt stress, DFF exhibited a very strong positive connection with 100-SW (0.430**). DM exhibited substantial positive association with PH (0.557***, 0.854***) and NSS (0.358*, 0.884***), under both control and salt stress conditions. Whereas under control condi-

Table 6: Simple phenotypic correlation co-efficient among seed yield and yield related traits of wheat genotypes in both control and salt stress condition.

		DM	PH	SL	SW	NSS	100-SW	GY
DFF	Control	0.446**	0.677***	-0.031	0.484**	0.831***	-0.304	0.543***
	Salt	0.639***	0.664***	0.111	0.740***	0.657***	0.430**	0.797***
DM	Control		0.557***	-0.468**	0.057	0.358*	-0.422**	0.117
	Salt		0.854***	-0.409*	0.914***	0.884***	0.779***	0.812***
PH	Control			-0.135	0.403*	0.708***	-0.186	0.515***
	Salt			-0.143	0.921***	0.778***	0.837***	0.911***
SL	Control				0.241	-0.060	0.579***	0.350*
	Salt				-0.127	-0.269	-0.049	0.026
SW	Control					0.682***	0.279	0.760***
	Salt					0.865***	0.842***	0.937***
NSS	Control						-0.235	0.717***
	Salt						0.729***	0.827***
100-SW	Control							0.176
	Salt							0.786***

Note: *, ** and *** indicates significant at 5%, 1% and 0.1% level of probability. Where, **DFF** = Days to first flowering, **DM** = Days to maturity, PH = Plant height, **SL** = Spike length, **PW** = Spike weight, **NSS** = Number of seeds per spike, **100-SW** = 100-seed weight, **GY** = Grain yield per spike.

tion, it exhibited a significant negative correlation with SL (-0.468**) and 100-SW (-0.422**). Under salt stress, DM revealed substantial negative correlation with SL (-0.409*) whereas positive correlation with SW (0.914***), NSS (0.884***), 100-SW (0.779***), and GY (0.812***). PH demonstrated a significant positive associations with SW (0.403*, 0.921***), NSS (0.708***, 0.778***), and GY (0.515***, 0.911***) under both control and salt stress, whereas it exhibited a significant positive correlation with SW (0.921***) and 100-SW (0.837***) under salt stress. Under control, SL exhibited significant positive correlations with 100-SW (0.579***) and GY (0.350*). Under both control and stress, SW exhibited significantly positive association with NSP (0.682, 0.865), and GY (0.760, 0.937), whereas exhibited a significantly positive connection with 100-SW (0.842***) under salt stress (Table 6). Under salt stress, NSS exhibited significant positive connection with GY (0.717***), but not with 100-SW (0.729***) or GY (0.827***). Under salt stress, 100-SW exhibited significant positive connection with GY (0.786***). Under both control and stress, GY demonstrated a significant positive correlation with DFF (0.543***, 0.797***), PH (0.515***, 0.911***), SW (0.760***, 0.937***), and NSS (0.717***, 0.827***), whereas under salt stress, it demonstrated a significant positive correlation with DM (0.812***) and 100-SW (0.786***). With GY only under control condition, SL demonstrated a considerable positive connection (0.350*) (Table 6). Notably, the positive correlation of GY with DFF, DM, PH, SL, SW, NSS, 100-SW under salt stress was supported by others (51, 52). These findings suggest the potential use of these traits in indirect selection to enhance yield. Furthermore, there was a slight variation in the relationships between the features under control and salt stress conditions. This suggests the need for different indirect selection techniques for rice genotypes grown in environments with or without salt stress (53)

Stress tolerance indices

Different stress tolerance indices, such as MP, GMP, SSI, TOL, STI and YSI values estimated based on seed yield per spike obtained from control and salt stress conditions, are presented in Table 7. The highest MP was obtained in the genotype of Binagom-1 (1.53), followed by BARI Gom 25 (1.34) and BARI Gom 20 (1.02). Similarly, the highest GMP was recorded in the genotype Binagom-1 (1.63), followed by BARI Gom 25 (1.15) and BARI Gom 20 (0.74). Considering SSI, the lowest value was observed in the genotype Binagom-1 (0.73), followed by BARI Gom 25 (1.07) and BARI Gom 20 (1.14). In accordance with the SSI value, the lowest value for TOL was found in the genotype Binagom-1 (0.68), followed by BARI Gom 20 (0.80) and BARI Gom 25 (0.97). The highest STI was obtained in the genotypes Binagom-1 (0.86), followed by BARI Gom 25 (0.60) and BARI Gom 20 (0.34). Similarly, the highest YSI was recorded in the genotype Binagom-1 (0.64), followed by BARI Gom 25 (0.47) and BARI Gom 20 (0.44). According to Rosielle and Hamblin (54) and Bouslama and Schapaugh (55), genotypes having higher values of MP, GMP, STI and YSI are considered resistant. Nevertheless, according to Krishnamurthy *et al.* (53), greater TOL and SSI values suggest relatively higher sensitivity to stress; thus, a lower TOL and SSI value for a particular genotype denote stronger stability of the genotype in stress and non-stress situations. Genotypes with high yields under stress conditions are favoured via selection based on these two parameters. According to MP, GMP, STI and YSI indices with high value and TOL and SSI with low values of these indices, Binagom-1 and BARI Gom 20 can be classified as salinity-tolerant varieties, whereas BARI Gom 20 was sensitive to salt stress. Importantly, stress tolerance indices clearly separated the tolerant and sensitive varieties, which are in accordance with the morphological and biochemical parameters conferring salt tolerance in wheat. Several other researchers also effectively identified salt-tolerant genotypes by using STI in wheat (10).

Table 7: Estimation of stress tolerance indices in wheat genotypes, estimated from grain yield per spike obtained in a control and salt stress condition.

Variety	MP	GMP	SSI	TOL	STI	YSI
BN 1	1.53	1.63	0.73	0.68	0.86	0.64
BR 25	1.34	1.15	1.07	0.97	0.60	0.47
BR 20	1.02	0.74	1.14	0.80	0.34	0.44

Here, **BN 1** = Binagom-1, **BR 25** = BARI Gom 25, **BR 20** = BARI Gom 20; **MP** = Mean Productivity; **GMP** = Geometric mean productivity; **SSI** = Stress susceptibility index; **TOL** = Tolerance index; **STI** = Stress tolerance index; **YSI** = Yield stability index.

Conclusion

Through a comprehensive analysis of the morphological and biochemical characteristics of tolerant and susceptible wheat seedlings, it was determined that root-shoot weight, shoot Na^+/K^+ ratio, chlorophyll, proline, methylglyoxal (MG), hydrogen peroxide (H_2O_2), and malondialdehyde (MDA) levels, as well as the activities of ascorbate peroxidase (APX), peroxidase (POD), and glyoxalase I (Gly-I), could all serve as potential morpho-biomarkers of salt tolerance. These markers have the potential to differentiate between susceptible and tolerant genotypes effectively. Furthermore, we refined a phenotypic technique tailored to the reproductive stages of wheat growth for categorizing tolerant and susceptible genotypes. This proposed reproductive-stage-specific phenotyping method may prove effective in identifying genes/QTLs conferring salinity tolerance at various stages of plant growth, thereby facilitating the development of a durable and salt-tolerant wheat variety. The results of correlation analysis revealed significant positive correlations between grain yield (GY) and days to first flowering (DFF), plant height (PL), spike weight (SW), and number of seeds per spike (NSS) under both control and stress conditions. However, there were significant negative correlations with days to maturity (DM) and 100-seed weight (100-SW) during salt stress conditions. Additionally, the outcomes of stress tolerance indices, including mean productivity (MP), geometric mean productivity (GMP), stress susceptibility index (SSI), tolerance index (TOL), salt tolerance index (STI), and yield stability index (YSI), were consistent with the phenotyping results. Nevertheless, further research is necessary

to identify additional crucial factors regulating salinity tolerance in wheat at different growth stages. This phenotyping method holds promise for identifying genes or QTLs that confer salinity resistance at distinct plant growth stages, thereby facilitating the development of a durable and salt-tolerant wheat variety.

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Authors' contributions

Conceptualization and methodology: SA, MAH; Experimentation and formal analysis: SA, SI, NC, SM K; Data analysis: SA, MMR; Original draft preparation: SA, SMK; Edited Table and Figure: SA and SMK; Writing, review and editing: MMR, LH and MAH; Supervision: LH and MAH; Project administration: MAH. All authors have read and approve the final version of the manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None.

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