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Improvement in wheat quality by expression of *Nax1* and *Nax2* genes under salt stress

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Abstract

The yield of wheat gets affected majorly due to high salt concentrations. Salinity among abiotic stress is the chief reason for the reduction in growth and development of plants. Agricultural land developing countries get affected by saline soil which limits the uptake of nutrients from the soils, results in decreased crop quality. The focus of present was to study the role of *Nax1* and *Nax2* genes under salt stress on the structural carbohydrates viz. NDF, ADF, cellulose, hemicellulose, lignin and silica in wheat. Role of *Nax1* and *Nax2* genes has been observed to protect the plants from the stress environment. Maximum ADF (43.5% in F₅ and 42.76% in F₆) and NDF (77% in F₅ and 68.8% in F₆) content under salt stress observed in the presence of both genes. Cellulose and hemicelluloses content reduced in the salinity environment and least reduction was observed in presence of sodium transporter genes *Nax1* and *Nax2* showing cellulose (34.5% in F₅ and 36.8% in F₆) and hemicelluloses (26.8% in F₅ and 26.1% in F₆). Lignin content also decreased under saline condition and maximum reduction observed in sensitive cultivar WH1105 (5.6% in F₅ and 4.5% in F₆) and presence of both Na⁺ transporter genes resist the reduction (8.4% in F₅ and 7.2% in F₆). Maximum increased in fodder quality was observed in the presence of both genes *Nax1* and *Nax2*.

Keywords: Cellulose, hemicelluloses, lignin, structural carbohydrates, wheat

Introduction

In coming years, to fulfill the demand of food for growing population becomes a big challenge as agricultural sector is facing a great loss in the yield due to salinity stress environment (Petronia *et al.*, 2011; Shabala *et al.*, 2013; Qadir *et al.*, 2014; Tack *et al.*, 2015; Abdel-Aal *et al.*, 2018) [17, 21, 18, 24, 2]. Salinity cover mostly cultivable land of arid and semi arid regions throughout world (Rostamza *et al.*, 2011; Shalaby, 2018; Borlu *et al.*, 2018) [19, 22, 5]. For securing the next generation from food scarcity, at present main focus of researchers is to produce a resistant variety which may overcome the adverse impact of stress on plant growth and development (Gilroy *et al.*, 2010; Abbasi *et al.*, 2012; Negrao *et al.*, 2017) [8, 1, 16]. Large portion of the wheat cultivable land under saline soil resulted in decreased yield both quantity and quality (Nabati *et al.*, 2011; Ashraf *et al.*, 2018) [15, 3]. To develop resistance in plants, various processes get upregulated, among them cross check on Na⁺ transporter is the primary concern under salt stress (Mudgal *et al.*, 2010) [12]. High-Affinity Potassium Transporters (HKT) restrict the uploading of Na⁺ ions from roots to shoot system (Munns and Tester, 2008; Munns *et al.*, 2010) [13, 14]. Na⁺ ions homeostasis under salt stress is maintained through the HKT genes viz. *Nax1* and *Nax2* (James *et al.*, 2006; Zhu *et al.*, 2015) [9, 35]. HD 2851 (salt sensitive) X Kharchia 65 (salt tolerant) a wheat cross under salt stress has been used to screen the existence of *Nax1* and *Nax2* genes which may play an important role in maintaining homeostasis of Na⁺ ions (Yadav *et al.* 2018) [33].

Cell wall is composed of mostly cellulose, lignin, hemicelluloses and proteins (Vogel, 2008). Cellulose, hemicellulose components of cell wall plays an essential role in protecting the plants from the drastic damage by stress (Bertrand *et al.*, 2006; Machinet *et al.*, 2011; Talbot and Treseder, 2012; Talbot *et al.*, 2012) [4, 11, 25, 26]. Lignin molecule stability depends on the extent of cross linking with hemicelluloses (Bertrand *et al.*, 2006; Talbot *et al.*, 2012) [4, 26]. To remove the impact of environmental stress on plants, breeding science plays major role in transforming the existing crop into resistant one against stress situation. Breeder focuses on increasing the photosynthetic ability which resulted in enhancing the biomass of crop plants, so that large amounts of agricultural residues used as animal fodder (Tanger *et al.*, 2015) [27].

Plant biomass majority determine by cell wall components like cellulose, lignin, hemicelluloses, pectins and proteins (Tao *et al.*, 2012) [28]. Present study was carried on a cross having Kharchia 65 that is tolerant to the salinity stress due to the presence of sodium transporter genes and WH1105 which is sensitive to salt stress but high yielding variety of wheat. Therefore, aim for producing this cross to having a variety which is high yielding in the presence of high saline environment.

Material and Method

Seeds of cross [Kharchia 65 (tolerant) / WH1105 (sensitive)] are obtained after confirming the resistance of plants against salinity by presence of *Nax1* and *Nax2* genes in F₂ (Dang Nguyen Luu Vi Vy, 2016) [32], F₃ (Kritika, 2017), F₄ (Yashveer, 2018) [33]. Two year experiment on F₅ and F₆ generation of cross [Kharchia 65 (tolerant) / WH1105 (sensitive)] of wheat (*Triticum aestivum* L. em Thell.) was conducted in the green house of College of Basic Science in 2016-2018 in collaboration with Department of Molecular Biology, Biotechnology and Bioinformatics. Plants expressing *Nax1* and *Nax2* genes were screened in F₅ generation by Varsha & Yashveer, (2018) [34] and in F₆ generation by Tiwari, (2019) [29] and the same plants were evaluated for structural carbohydrate to determine the quality of wheat under saline condition. F₅ plants were grown at the 80 mM salt level and F₆ generation was grown at 100 mM salt level as stability was attained in advanced generation and higher level that is 100 mM was tested to screen the salinity tolerance. Samples were collected at maturity stage of crop and was dried in hot air oven for 3-4 days at 100°C, after that dried sample was grinded. Grinded sample was further used to estimation the structural carbohydrate *viz.* neutral detergent fiber, acid detergent fiber, cellulose, lignin, silica, and hemicelluloses (Van Soest, 1991) [30].

Neutral detergent solution (NDF)

NDF was estimated by refluxing one gram of dried sample for one hour in 100 ml neutral detergent solution containing 500 mg of sodium sulphite on refluxing apparatus. Use the preweighed (W₁) Gooch crucible (G-1) to collect the extract by washing with hot distilled water under vacuum and final washing with acetone (W₂). Dry the extract in hot air oven for overnight at 100°C and weigh again (W₃).

Neutral detergent fibre (% dry wt. Basis) = $W_2 - W_1 / \text{weight of sample} \times 100$

Acid detergent solution (ADF), Cellulose, Lignin, Silica

ADF, cellulose, lignin, silica were estimated by refluxing one gram of dried sample was added in 100 ml of acid detergent solution in berzelius beaker on refluxing apparatus. Use the preweighed (W₁) Gooch crucible (G-1) to collect the extract by washing with hot distilled water under vacuum and final washing with acetone Dry the extract in hot air oven for overnight at 100°C and weigh again (W₂). Sulphuric acid (72%) poured in crucible with constant stirring with glass rod and kept at room temperature for three hours. Sulphuric acid was removed from crucible with hot distilled water. To dry the sample kept crucible in hot air oven for overnight after that cool them weighed the crucible and marked them as (W₃). For next 3 hours, ignite the extract at 550°C in muffle furnace. Followed day, crucible was cooled in dessicator and weighed the crucible and marked them as (W₄).

Acid detergent fibre (% dry wt. Basis) = $W_2 - W_1 / \text{weight of sample} \times 100$

Cellulose (% dry weight basis) = $W_2 - W_3 / \text{Weight of sample} \times 100$

Lignin (% dry weight basis) = $W_3 - W_4 / \text{Weight of sample} \times 100$

Silica (% dry weight basis) = $W_4 - W_1 / \text{Weight of sample} \times 100$

Hemicellulose content

Difference of ADF % from NDF % was the hemicellulose content in the sample.

Statistical Analysis

The data was analyzed by OP STAT for the complete randomized design (CRD) with critical difference (P= 0.05).

Results and Discussion

To study the impact of sodium ion transporter genes *Nax1* or *Nax2* on wheat quality Structural carbohydrates of advanced lines of wheat cross [Kharchia 65 (tolerant) / WH1105 (sensitive)] were estimated. Sodium ion unloading is considered as the primary step taken by plants to avoid the harmful effect of salt stress on their growth and development (Saddiq, 2019).

Kumar *et al.* (2018) [10] studied the alteration of structural carbohydrate content in *Dichanthium* under salt stress, notified that all component of structural carbohydrate except NDF get affected significantly. They found that highest NDF content was observed in the control condition sample whereas salt stressed plants sample observed with reduced NDF. Xu & Lascano (2007) and Seif *et al.* (2016) [20] found that in corn with higher level of stress there was increased in the ADF and NDF content which reduced the quality of crop. Seif *et al.* (2016) [20] showed that KSC705, had the least increase in ADF and NDF content. In present experiment, reduction in NDF content (Figure 1 A) as in stress condition, Kharchia 65 was 20.5% F₅, 26.45% in F₆ and in WH1105 was 29.7% F₅, 34% in F₆ when compared to control parent. HKT transporter genes help to resist the change due to stress environment which was also observed in present experiment such that presence of both genes *Nax1* and *Nax2* resist the reduction and increased NDF content by 9.07% in F₅ and 5.31% in F₆ in contrast to tolerant variety, Kharchia 65 in stress condition. Alone presence of either genes also limit the reduction rate upto some level i.e. *Nax1* only gene recorded 5.52% in F₅ and 0.6% in F₆ NDF content whereas only *Nax2* gene showed lesser protective in defending alteration in structural carbohydrate against salinity in as compare to *Nax1* gene.

Similar trend was observed for ADF content (Figure 1 B) i.e. *Nax1* and *Nax2* genes helps the plants to resist the change due to salt stress so there was less reduction was observed. ADF content was decreased in salt stress. In present study, ADF content in tolerant variety Kharchia 65 in stress reduced by 4.65% in F₅, 7.8% in F₆ from the controlled conditions. The plants of cross with the expression of both *Nax1* and *Nax2* genes helps the plants to decrease the effect of salinity and there were increased in ADF under both genes presence by 0.9% in F₅, 2.2% % in F₆ as compared to Kharchia 65 in stress. The individually presence of either genes helps the plants to protect the plants from changes in the structural carbohydrate but to a lower extent as compare to the presence of both *Nax1* and *Nax2* genes.

Cellulose content is the chief component of the cell wall which plays important role in protecting against any stress

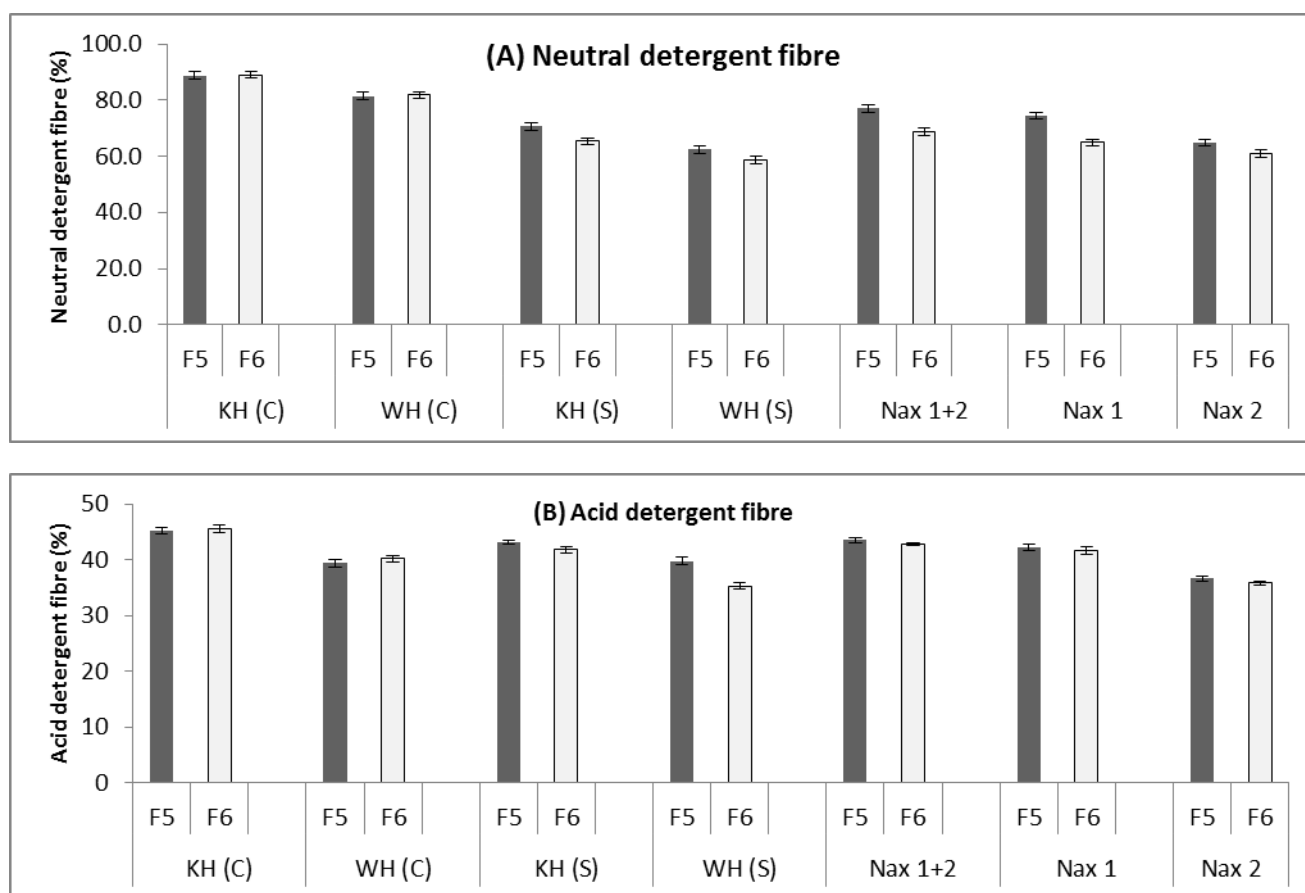
environment. In present study, cellulose content (Figure 1 C) was increased in the stress environment which was maintained with the help of *Nax1* and *Nax2* genes. The range of cellulose content was observed Kharchia 65 (15.53% in F₅, 20.13% in F₆), WH1105 (5.18% in F₅, 9.26% in F₆) in saline condition as comparison to controlled environment. Plants with presence of gene *Nax1* and *Nax2* was recorded cellulose content (3.64% in F₅ and 0.92% in F₆), followed by only *Nax1* (5.32% in F₅ and 2.24% in F₆) and then *Nax2* (7.96% in F₅ and 9.87% in F₆) as comparison to salt stressed Kharchia 65 plants.

Lignin content (Figure 1 D) is second chief component of cell wall after cellulose. Salt stressed plants have the reduced level of structural carbohydrates in coffee plant due to which cell wall of the plants get thinner (De Lima *et al.*, 2014) [7]. Observed results for lignin content in present study depict that there was decreased in level of lignin content as stress level increased. F₆ generation at 100 mM NaCl mediated stress showed less amount of lignin as compared to the F₅ generation which was grown at 100 mM NaCl mediated stress.

Reduction in lignin content showed by Kharchia 65 (39.54 in F₅ and 45.81% in F₆) and WH1105 (34.88% in F₅ and 48.02% in F₆) as compared to the parent plants grown under controlled condition. In contrast to Kharchia 65 plants in

stress conditions, plants having *Nax1* and *Nax2* genes resist the reduction by 61.54% in F₅ and 53.19% in F₆ showed lesser reduction in the lignin content whereas presence of *Nax1* (27% in F₅ and 30.2% in F₆) or *Nax2* (34.6% in F₅ and 34.9% in F₆) gene individually. Higher reduction was measured in F₆ generation as compared to F₅ generation because of higher level of salt stress.

Digestibility factor strictly affected by the presence of structural carbohydrates like cellulose, hemicelluloses, lignin. Similar trend as cellulose were observed for hemicelluloses and ash content (Figure 1 E, F). Reduction in hemicellulose and ash content were observed in salt tolerant variety Kharchia 65 (7.37% in F₅ and 11.23% in F₆), (50% in F₅ and 59% in F₆) respectively s compared to Kharchia 65 plants grown in controlled conditions and maximum reduction in the loss was observed in plants having both *Nax1* and *Nax2* genes (1.52% in F₅ and 3.16% in F₆), (18.18% in F₅ and 11.11% in F₆) respectively in contrast to Kharchia 65 plants in salt stress. Chakravarthi, (2017) [6] stated that there is strong correlation between the reduction in hemicelluloses content and increased proportion of ADF. Presence of sodium transporter genes helps plant to unload the accumulated excess of ions in leaf and activate the mechanism which limits the reduction in quality of wheat under unfavorable conditions.



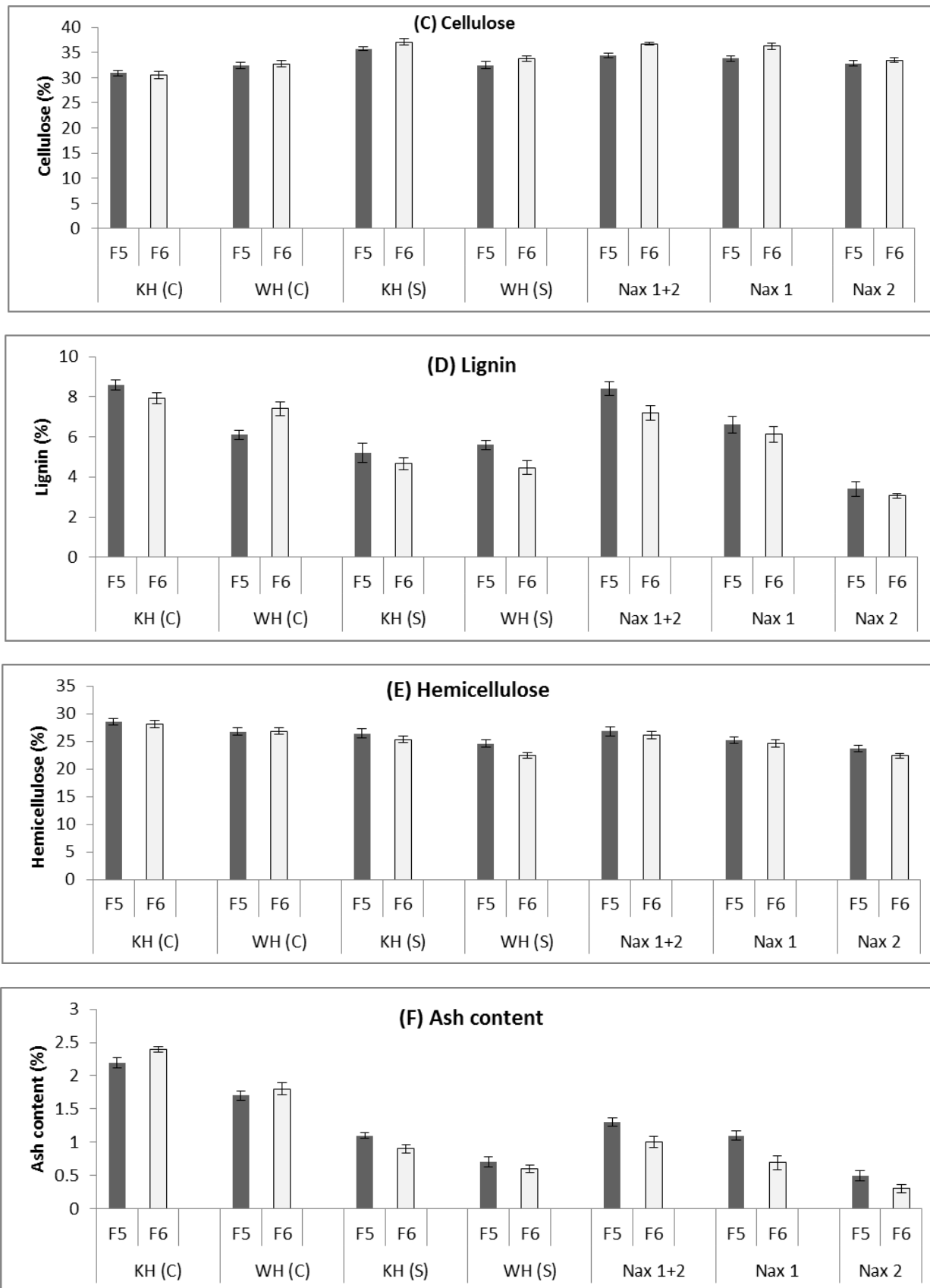


Fig 1: Effect of genes *Nax1* and *Nax2* on structural carbohydrates (A) neutral detergent fibre, (B) acid detergent fibre, (C) cellulose (D) lignin (E) hemicelluloses and (F) ash content under F₅ at 80 mM and F₆ at 100mM NaCl mediated salt stress.

*In figure

- KH (C) = Kharchia 65 in controlled condition
- WH1105 (C) = WH1105 in controlled condition
- KH (S) = Kharchia 65 in stress condition
- WH1105 (S) = WH1105 in controlled condition
- Nax 1+ 2 = Presence of both gene *Nax1* and *Nax2*
- Nax1 = Presence of solely *Nax1* gene
- Nax2 = Presence of solely *Nax2* gene

Conclusion

Role of sodium transporter genes were clearly observed in strengthen the plant against salt stress. Mass production of yield are lesser affected under the influence of *Nax1* and *Nax2* genes by protecting the huge alteration in structural carbohydrates.

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