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MOLECULAR AND MORPHOLOGICAL EVALUATION OF SALT TOLERANCE GENES IN ADVANCED WHEAT LINES (PART 1)

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Abstract

Soil salinity is a key problem in cereal crops production that affects the metabolic activities as well as plant growth ultimately leading to plant death. Toxic effect of salinity can lead to reduced plant growth and yield. To counter these effects, tolerant varieties need to be developed that should be able to survive in saline conditions. Therefore, experiment was designed to evaluate yield and yield attributes of 29 advanced wheat lines with four check varieties in Randomized Complete Block Design (RCBD) with three replication. The research was conducted at National Agriculture Research Centre (NARC), Islamabad in 2013-2014 winter season. These lines have been bred for salt 'tolerant' traits in view to bring saline soils under wheat cultivation. The eleven morphological parameters studied in this experiment were significant at ($p \leq 0.05$). An effective methodology was adopted for breeding programme to screen out genes that showed response to abiotic stresses using DNA markers. It was concluded from the correlation study that grain yield (kg/hectare) was significantly positive correlated with plant height (0.1802) but showed non-significant negative correlation with spike length (-0.1818), number of infertile spikelets per spike (-0.2352*), number of fertile spikelets per spike (-0.1121), and the number of fertile tillers, 1000 grain weight was significantly positive correlated with peduncle length (0.0019,), number of grains per spike (0.0823), grain yield per spike (0.0416) and showed negatively non-significant correlation with leaf area (-0.1357), number of infertile spikelets per spike (-0.1207), number of fertile spikelets per spike (-0.1523) and number of fertile tillers (-0.3135*).Morphological and molecular data confirmed that among all the 29 advanced wheat lines PA06B, PA02K, PA03B, PA02G and PA03C had the high potential for all the morphological parameters linked to the SSR markers. Five advanced wheat lines were selected on the basis of their high yield potential and its components and DNA markers linked to these morphological parameters compared with check varieties. Results of cluster analysis based on microsatellite data showed that the five genotypes were located in close proximity to each other in the same cluster but not clearly separated from the remaining 24 genotypes and check cultivars were also closely linked to these advanced wheat lines, all of which had been collected from the same habitat.

Key words: Wheat, Polymorphism, Linkage, Salt tolerance and Advance lines.

Introduction

Wheat (*Triticum aestivum* L.) is one of the main food crops with a production of 750 million tons annually (2016–2018, Anon., 2019) and has become the second most important crop after maize (http://www.fao.org/worldfoodsituation; Murdia *et al*., 2016). Wheat production in Pakistan is expected to be 27 million tonnes in the 2021-22 marketing year, an 8% increase over the 25 million tonnes produced in the previous year 2019-2020 (Anon., 2021). Wheat yield is hampered by many factors including salinity stress. About 800 mha (6%) is affected by either salinity (397 mha) or sodicity (434 mha) worldwide (Anon., 2005). Demand of wheat will be about 1 billion tons in the year 2020 which is almost double as compare to 1990s (Rosegrant, 1997).

Salinity is one of the major stresses which affect the yield of many crops. Salt tolerance level vary among different plant genera and species (Shahbaz & Ashraf, 2013). Salinity stress disturbs the functionality of normal ion channels, homeostasis and osmotic potential processes of the plant. Other important processes such as photosynthesis, respiration, and transpiration are highly affected by excess salt conditions in many important crop species including wheat (Zhu, 2001; Munns, 2005; Ahmad *et al*., 2011). The salt stress reduces root pressure because of the higher ions concentration in the rhizosphere (Khan *et al*., 2001).

To improve the crop plant for salt tolerance, it is a major step to elucidate the mechanisms of salt tolerance. It can lead us to find out genes responsible for salinity tolerance in plants (Liang *et al*., 2018). Salinity affects various plant phenomena but at cellular level it can cause the inhibitory effect on the cell division retard to normal growth (McCue and Hanson, 1990). Toxic effect of salinity can lead to reduce the yield by affecting early flowering, root and shoot size, leaf surface area and also the enzymatic activities in the cell (Rawson, 1986; Mass & Poss, 1989; Francios *et al*., 1986). It has been reported that high evaporation rate of a plant can increase the high salt uptake in leaves which kill the leaf tissues before maturity (Dodd & Donovan, 1999). Salt stress adversely affect the photosynthetic rate primarily through a reduction in chlorophyll content, leaf area, stomatal regulation as well as photosystem II functionality (Netondo *et al*., 2004).

The higher level of salt concentrations in tissues decrease normal morpho-physiological and molecular processes (Munns & Tester, 2008; Keshavarz & Karami, 2013). Higher K^+ concentration supports plant growth and reduce salt effect (Safaa *et al*., 2013). The response of

different plants and their tissues toward salinity is highly variable, which depends upon the type and the age of tissue and its importance in plant organization. If the plant is capable of restoration of homeostasis, balancing ions concentration and regulating metabolic pathways with appropriate water uptake which are disturbed by salinity, can survive and grow in saline conditions (Saqib *et al*., 2005; Tahir *et al*., 2006; Romero *et al*., 2006).

Salt tolerant plants normalize the Na^+ and K^+ ion concentration which leads to opening and closing of stomata, and osmosis in different tissues of the plant (Liang *et al*., 2003; Yeo *et al*., 1999; Liang *et al*., 2005; Tahir *et al*., 2006; Hasegawa *et al*., 2000). Normal physiological functions and optimum uptake of nutrients in salt stress condition is also reported as a reason of tolerance (Murillo *et al*., 2007; Matoh *et al*., 1986). Generally, a soil salinity of 0.2 to 0.5% harmfully affects plant growth; however, the soil surface salinity in saline areas usually ranges from 0.6 to 10%. Ion poisoning arises from two different processes. The influx of large amounts of Na^+ into plants increases the Na^+ content, which reduces or deactivates the activity of several enzymes (Pan *et al*., 2008). The normal Na^+ ion is very important in salt tolerant plants because these plants keep Na^+ ion in balance which is required for many biological activities (Tahir *et al*., 2006).

In Pakistan, salinity is a major problem to many crop plants that reduces their growth and yield up to several folds. Plant tolerance to salinity differs at different growth stages. Therefore, it is necessary to carry out the assessment of wheat at different growth stages. Salt stress affects the plants at all growth stages but germination and seedling stages are more susceptible for salinity. Ion stress also affects some plants at germination and seedling stages (Catalan *et al*., 1994; Carvajal *et al*., 1998). Grain yield is reduced more than vegetative growth by salt stress. Genotypes are found less sensitive to salt stress at germination than after the three-leaf stage of growth.

Utilization of DNA markers in genetic variations study and QTL mapping in various crops species for salt tolerance have been previously reported by many researchers (Ma *et al*., 2007; Kurup *et al*., 2009). But the implication of SSR to investigate genetic variation with respect to salt tolerance in Pakistani wheat landraces/cultivars is meager and limited. Quantitative trait Loci mapping approach being one of the new emerging technologies in dissecting complex traits into genomic components, taking part in expression of that trait, promises a lot in the future for wheat improvement (Reynolds and Borlaug, 2006). DNA markers are also used in determining the phylogenetic analysis and to find out the genetic relationships in plants. Other than gene mapping, these markers are extensively used in the study of genetic characterization, genome organization and cultivars identification (Bhutta *et al*., 2006). Random amplified polymorphic DNA (RAPD) and simple sequence repeats (SSRs) are used to identify many genes including the stress tolerant gene and also to improve yield in salt affected fields. Simple sequence repeats (SSRs) are PCR-based markers, and generally they have the distinguishing features of high information content, simplicity, codominance, even distribution throughout the genome, and locus specificity (Zhang *et al*., 2008). Improvement of wheat varieties having salt tolerance potential using conventional breeding methodologies is difficult as salinity tolerance is controlled by multiple genes. The use of genetic and genomic analysis to identify DNA regions firmly linked to quantitative traits in crops, called molecular marker-assisted breeding, can make possible breeding programmes for wheat improvement through indirect selection and reducing the time required to reduce segregation (Munns & Tester, 2008). The present research was conducted to characterize salt tolerant genotypes with the help of SSR markers and to evaluate the morphological performance of these genotypes in field conditions.

Materials and Methods

Plant materials: Grains of 33 advanced wheat lines including four check varieties were acquired from National Institute for Genomics and Advanced Biotechnology (NIGAB), National Agriculture Research Centre (NARC), Islamabad. All the wheat genotypes were sown in 3 replicates in the field at NARC. These lines were bred for enhanced salt tolerance and were tested for salt tolerance at two locations for two years in an experiment conducted along with the experiment described. The lines screened out were advanced to F7 (Ahmad *et al*., 2011). The purpose of the present study was to conduct preliminary wheat yield trials and to screen these lines with DNA markers for salt tolerance traits.

Sowing method: The seeds were sown in well prepared field at National Agriculture Research Centre (NARC), Islamabad during the crop growing seasons 2013-14. Randomized Complete Block Design was followed with three replications keeping row to row distances 30 cm along with row length of 4 meter.

Evaluation of morphological characters: Plant height of central spike of randomly selected three plants was measured in cm from ground to the apex of spike. Number of tillers per plant was counted of each row in every replication. Leaf area (cm^2) was calculated at maturity from three replicates. Peduncle length (cm) was measured at maturity from each replication. Spike length (cm) from 3 randomly selected spikes was taken from base to top of spike from each repeat. The data of 3 spikes was averaged. The number of days to reach the growth stage when half of the spike was visible out of flag leaf (spike initiation) was recorded. The number of fertile spikelet spike per spike was calculated from spike of main tiller of each selected plant at maturity from three replicates. The number of infertile spikelet spike per spike was calculated from spike of main tiller of each selected plant at maturity. The number of grains spikeper spikewas calculated from spike of main tiller of each selected plant at maturity. The crop was harvested at maturity and threshed. Grain yield per spike was recorded in grams with the help of electrical balance (Unibloc, shimadzaATY224). Grain yield was recorded from one line of 4m from each repeat in gms with the help of

electric balance and then converted into (kg ha^{-1}) multiplied with the factor 6.25. Thousand grain weights in gms was recorded for each line in three repeats.

DNA extraction: Young leaf samples were collected from 2-3 weeks old plants and immediately stored at -80°C. These samples were processed for DNA isolation using modified CTAB method following Doyle & Doyle (1987). Around 0.2 gm of leaf samples were crushed in liquid nitrogen using mortar and pestles. After crushing, the leaf samples were processed with the 750 µl pre-warmed extraction buffer (CTAB) having 1% β-mercaptoethanol and incubated at 65°C for 30 min. After the addition of 0.8 volume of chloroform-isoamyl alcohol (24:1), these tubes were centrifuged at 12000 rpm for 10 minutes at 4°C. After centrifugation, 600 µl aqueous layers were treated with 100 µl sodium acetate and 600 µl of isopropanol and incubated at -20°C for at least 20 min. After centrifugation at 12000 rpm for 10 minutes at 4°C, supernatant was discarded and pallet was washed with 200 µl wash solution (70% ethanol). This step was repeated twice and then air dried for overnight at room temperature. The pellet was re-suspended in 50µl double distilled water and treated with RNAse (10 mg/mL).

DNA quantification: The DNA quantification was determined by comparing the bands intensity with lambda DNA standard of known concentration with 1.5% agarose gel stained with 0.6µl/ml ethidium bromide. Gel electrophoresis was carried at 100V for 30 min. DNA bands were visualized under UV light at 260 nm and photographed using a gel documentation system. Quantified DNA samples were diluted using following equation to working concentration of 25 ng/µl.

$$C1V1 = C2V2 \ldots\ldots\ldots\ldots\ldots (1)$$

where
C1 = Stock concentration (ng/µl)
V1 = Volume to be taken from stock
C2 = Required working concentration (ng/µl)
V2 = Total volume of the required working stock

Primer ordering and dilution: Total of 23 SSR markers were used in the current study as shown in Table 2. Primer sequences were obtained from previously reported paper and wheat genome database (Moolhuijzen *et al*., 2007) and were synthesized by e-oligos. These markers were selected on the basis of their linkage (appendix-I) with salt stress tolerance in wheat (Shahzad, 2007). Primers were diluted with RNAs free water according to primer magnitude "nmol" × 10 to prepare 100pmol/µl stocks. Primers were diluted to working concentrations according to equation known in section 3.5.

PCR analysis: PCR amplifications were performed according to the method of Roder *et al*., (1998) with some modifications. PCR reactions were carried out in 20 µL volume in Veritii 96 wells thermal cycler (Applied Biosystems). PCR mixture contained 25ng of wheat genomic DNA, 20 pmol of each primer, 0.4 M dNTP mix, 2.5 µM $MgCl_2$, 10X PCR buffer, and 1 unit of Taq DNA polymerase (Thermo Scientific). The PCR cycling reactions were hot started for 5 min at 95°C. Thirtyfive cycles were performed as follows: 1 min at 94°C, 1 min at 58°C, 1 min at 72°C, and a final extension step for 10 min at 72°C as shown in Table 1. All the 23 SSR markers were optimized at the same condition.

Gel electrophoresis: PCR products were separated on 2.5% denaturing agarose gel and with 0.6µl/ml ethidium bromide in 1XTBE buffer. Electrophoresis was applied at 100V for 35-40 min. After electrophoresis, DNA bands were visualized under UV light and photographed using a gel documentation system. Bands were scored for screening of wheat genotypes. The manufacturers of gel documentation systems were Bio-Rad.

Statistical analysis

All the morphological data were measured regarding all the traits at the plant maturity level by ANOVA in order to see the significance of genotype at morphological level. The analysis of data was done using computer-based software Minitab 16 for Windows: Advance statistic, 1994. Molecular marker data was used to assess genetic diversity in 33 wheat genotypes. Presence of each allele of a marker was scored as "1" and absence of an allele was scored as "0". The data matrix was analyzed in NTSYS Pc V 2.1. Ni's and Li's coefficient was used to assess similarity among wheat genotypes. The similarity coefficients were clustered using UPGMA and graphed using tree plot function.

Table 1. PCR master mix (20 µl rxn).

S. No.	Component	Stocks	Final conc.	1 rxn (µl)	35 rxn (µl)
1.	10 × PCR buffer	10 X	1X	2	70
2.	MgCl2	25 mM	3 mM	2.4	84
3.	dNTP mix	10 mM	0.2 mM	0.4	14
4.	Primer forward	100 pM	20 pM	1	35
5.	Primer reverse	100 pM	20 pM	1	35
6.	ddH_2O	---------	----------	12	420
7.	Taq polymerase	5 U/µl	1 U/rxn	0.2	7
8.	DNA template	25 ng/µl	20 ng/rxn	2	2

Conc. = Concentration

Table 2. List of all the SSR markers along with forward and reversed primer sequences.

Marker	Forward primer	BP	Reverse primer	BP
gwm357	5' TATGGTCAAAGTTGGACCTCG 3'	21	5' AGGCTGCAGCTCTTCTTCAG 3'	20
gwm18	TGGCGCCATGATTGCATTATCTTC	24	GGTTGCTGAAGAACCTTATTTAGG	24
gwm11	GGATAGTCAGACAATTCTTGTG	22	GTGAATTGTGTCTTGTAT GCTTCC	24
cfd19	5' TACGCAGGTTTGCTGCTTCT 3'	20	5' GGAGTTCACAAGCATGGGTT 3'	20
gwm148	GTGAGGCAGCAAGAGAGAAA	20	CAAAGCTTGACTCAGACCAAA	21
gwm539	5' CTGCTCTAAGATTCATGCAACC 3'	22	5' GAGGCTTGTGCCCTCTGTAG 3'	20
gwm674	5' TCGAGCGATTTTTCCTGC 3'	18	5' TGACCGAGTTGACCAAAACA 3'	20
gwm383	5' ACGCCAGTTGATCCGTAAAC 3'	20	5' GACATCAATAACCGTGGATGG 3'	21
gwm533	AAGGCGAATCAAACGGAATA	20	GTTGCTTTAGGGGAAAAGCC	20
gwm645	5' TGACCGGAAAAGGGCAGA 3'	18	5' GCCCCTGCAGGAGTTTAAGT 3'	20
gwm2	CTG CAA GCC TGT GAT CAA CT	20	CAT TCT CAA ATG ATC GAA CA	20
gwm133	5' ATCTAAACAAGACGGCGGTG 3'	20	5' ATCTGTGACAACCGGTGAGA 3'	20
gwm205	5' CGACCCGGTTCACTTCAG 3'	18	5' AGTCGCCGTTGTATAGTGCC 3'	20
Gwm335	5' CGTACTCCACTCCACACGG 3'	19	5 CGGTCCAAGTGCTACCTTTC 3	19
Gwm371	5' GACCAAGATATTCAAACTGGCC 3'	22	5' AGCTCAGCTTGCTTGGTACC 3'	20
Gwm604	5' TATATAGTTCAATATGACCCG 3'	21	5' ATCTTTTGAACCAAATGTG 3'	19
Gwm174	5' GGGTTCCTATCTGGTAAATCCC 3'	22	5' GACACACATGTTCCTGCCAC 3'	20
Gwm159	5' GGGCCAACACTGGAACAC 3'	18	5' GCAGAAGCTTGTTGGTAGGC 3'	20
cfd49	5' TGAGTTCTTCTGGTGAGGCA 3'	20	5' GAATCGGTTCACAAGGGAAA 3'	20
gwm121	5' TCCTCTACAAACAAACACAC 3'	20	5' CTCGCAACTAGAGGTGTATG 3'	20
gwm332	5' AGCCAGCAAGTCACCAAAAC 3'	20	5'AGTGCTGGAAAGAGTAGTGAAGC 3'	23
psp3113	CTCTCAAAGCCGCCACCATAGTC	22	CGTCGCTTACCGGTCCTGTCC	21
gwm111	5' TCTGTAGGCTCTCTCCGACTG 3'	21	5' ACCTGATCAGATCCCACTCG 3'	20

Table 3. Twenty-nine advanced wheat lines with four check varieties used in the present study.

S. No.	Genotype	S. No.	Genotype
1.	PA02C	18.	PA06H
2.	PA06G	19.	PA02E
3.	PA07B	20.	PA02B
4.	PA06D	21.	PA03C
5.	PA06J	22.	Faisalabad-08*
6.	PA02D	23.	Sehar-06*
7.	PA02G	24.	PA03F
8.	PA02H	25.	PA07A
9.	PA06C	26.	PA02J
10.	PA02K	27.	PA06I
11.	PA02I	28.	PA03E
12.	PA02G	29.	PA06A
13.	PA03A	30.	PA06F
14.	PA06E	31.	PA02A
15.	PA06B	32.	NARC 2009*
16.	PA02F	33.	NARC 2011*
17.	PA03D		

*Commercial check varieties

Results and Discussion

Evaluation of molecular characteristics of advanced wheat lines: Twenty-three SSR markers were used to determine presence or absence of salt tolerance related traits in 33 different advanced wheat lines (Tables 2 & 3). The results showed that out of 33 lines, 26 lines including check varieties were positive (193 bp band) for this marker, while only 7 lines could not amplify the targ*et al*lele (Fig. 1 a&b). Xgwm159 was linked with trait K^+ accumulation at 200 mM salt stress on the chromosome 5D (Shahzad, 2007). Xgwm533 marker produced the targ*et al*lele of 130 bp in most of the genotypes and the product size showed variation in different lines. Nineteen advanced wheat lines including Sehar-06, NARC-2009 and NARC-2011 were positive for the fragment of 130 bp and remaining lines were negative for this trait (Fig. 1 c&d). In a previous research study, Xgwm533 marker showed the presence of Sr2 gene complex for rust resistance on chromosome 3B (Hayden *et al*., 2004). Xgwm174 were used to detect the salt tolerant traits in all the 33 advanced wheat lines. Almost all the genotype showed variation to the expected band size. Seventeen genotypes showed the presence of 200 bp including the check variety Faisalabad-08 and remaining 17 lines including the Sehar-06, NARC-2009 and NARC-2011 check varieties were positive for the 400 bp product size (Fig. 1 e&f). Ahmad *et al*., (2013) in their study reported that the amplified fragment size of Xgwm174 was 400 bp which were linked to the trait of biomass production and grain yield of the wheat under salt stress on chromosome 5D. During this research study, all of the advanced wheat lines showed polymorphic bands such as 130 bp product size for Xgwm121 (Fig. 1 g&h). Xgwm121 is a co-dominant marker, which is linked to the spikelets sterility trait on the 7A chromosome (Shahzad, 2007). Similar result was reported by the Quarrie *et al*., (2005) that a QTL for grain yield in wheat under a range of environment on the same chromosome closely linked to gwm-121.

Table 4. Mean performance and standard deviation of 33 advanced wheat lines with four check varieties.

Genotypes	PH	SL	PL	LA	NGPS	NIS	NFS	GWPS	NFT	GY	SI	1000 GW
PA02C	105.6 ± 5.51	13 ± 1.00	35 ± 1.00	52 ± 6.00	57.33 ± 3.2	3 ± 2.05	21.33 ± 2.52	1.973 ± 0.61	429 ± 349	2670 ± 140.6	105 ± 0.58	33.93 ± 4.05
PA06G	98.3 ± 0.58	12.33 ± 0.58	35.33 ± 1.16	48.33 ± 8.08	52 ± 3.61	2 ± 0.05	22.33 ± 0.58	1.277 ± 0.20	414 ± 238	1442.75 ± 153.8	106 ± 0.5	24.45 ± 2.21
PA07B	80.67 ± 5.51	11.67 ± 0.58	28 ± 2.46	37 ± 8.89	53.33 ± 5.13	2.66 ± 1.53	19.33 ± 1.52	1.66 ± 0.380	275 ± 171	1667.5 ± 350	110 ± 0.50	31.07 ± 6.47
PA06D	97.67 ± 3.79	14 ± 1.00	37 ± 2.65	59 ± 3.00	35.33 ± 8.62	2 ± 0.05	21.67 ± 1.15	0.917 ± 0.215	329 ± 404	1205.9 ± 127.7	103 ± 2.08	25.9 ± 1.47
PA06J	96 ± 8.72	12.667 ± 0.58	32.33 ± 1.53	52.33 ± 6.66	54.67 ± 0.58	3 ± 1.00	23.00 ± 1.00	1.4133 ± 0.11	376 ± 269	2421.5 ± 276.8	107 ± 1.53	25.84 ± 1.71
PA02D	101.33 ± 1.15	13 ± 0.05	37.67 ± 1.53	52.33 ± 1.53	51.67 ± 2.52	2.33 ± 1.52	21.33 ± 1.15	1.593 ± 0.175	523 ± 342	1863.25 ± 768.9	109 ± 0.60	30.84 ± 3.06
PA03B	141 ± 7.00	12.67 ± 0.58	47 ± 2.46	55 ± 11.79	46 ± 2.00	2 ± 0.00	20.33 ± 0.58	1.94 ± 0.05	390 ± 211	4509.5 ± 697.2	105 ± 1.53	42.24 ± 2.61
PA02H	107.3 ± 4.93	13 ± 0.05	48 ± 2.57	54.33 ± 5.03	48.67 ± 6.11	1.66 ± 0.57	20.67 ± 1.53	1.8633 ± 0.14	265 ± 224	2549.6 ± 429.18	103 ± 1.73	38.47 ± 2.35
PA06C	97.67 ± 2.31	10.67 ± 0.58	33 ± 2.00	42.33 ± 7.77	47.33 ± 4.04	1.33 ± 0.57	19.67 ± 0.58	1.4633 ± 0.08	583 ± 170.8	3418.4 ± 425.5	101 ± 0.70	31.06 ± 3.14
PA02K	92 ± 7.21	11.33 ± 0.58	34.33 ± 2.04	47 ± 1.000	67 ± 3.00	1.33 ± 0.57	21.00 ± 1.00	2.40 ± 0.0557	520 ± 304	4611 ± 597.6	103 ± 1.53	35.85 ± 1.23
PA02I	86.33 ± 8.08	12.33 ± 0.58	33 ± 1.73	45.67 ± 5.51	50 ± 4.58	3 ± 1.00	19.00 ± 3.00	1.20 ± 0.219	461 ± 333	1460.8 ± 597.6	107 ± 1.15	24.48 ± 6.87
PA02G	134.6 ± 7.77	12.33 ± 1.16	49 ± 2.57	47.33 ± 7.64	52.67 ± 1.53	2 ± 0.05	19.67 ± 0.58	2.12 ± 0.05	419 ± 58.2	4455 ± 66.6	105 ± 2.08	40.32 ± 0.48
PA03A	122.3 ± 19.7	12 ± 1.00	54 ± 2.46	50 ± 3.46	41 ± 5.57	2.66 ± 1.52	18.00 ± 1.00	1.78 ± 0.284	371.3 ± 56.6	2653.5 ± 256	111 ± 0.50	43.33 ± 1.34
PA06E	106 ± 31.6	12 ± 0.07	31.67 ± 3.51	43.33 ± 0.58	45 ± 6.56	2 ± 1.00	21.67 ± 1.52	1.03 ± 0.075	495 ± 98.5	1834.25 ± 369	101 ± 0.50	23.27 ± 3.44
PA06B	97 ± 4.58	11.333 ± 1.16	34.33 ± 2.89	43.33 ± 3.79	48.67 ± 6.35	1.66 ± 1.15	17.67 ± 3.21	1.79 ± 0.255	646 ± 126	5310.6 ± 374.2	101 ± 0.60	16.02 ± 3.55
PA02F	94.67 ± 2.52	12.333 ± 0.58	33.67 ± 0.58	50.33 ± 6.03	53.67 ± 6.5	2.66 ± 1.52	20.33 ± 2.08	1.47 ± 0.665	294 ± 133.3	2639 ± 133	108 ± 0.50	26.35 ± 6.83
PA03D	123 ± 9.85	11.667 ± 1.16	48 ± 1.00	49.33 ± 8.50	53 ± 5.29	1.33 ± 0.57	20.67 ± 1.16	2.01 ± 0.393	241.7 ± 64.5	2805.75 ± 399.8	104 ± 1.00	37.84 ± 4.79
PA06H	106 ± 4.58	13 ± 1.000	37.33 ± 2.08	48 ± 1.000	49.6 ± 6.01	1.66 ± 0.57	21.67 ± 0.58	1.29 ± 0.181	828 ± 341	1131 ± 41.01	104 ± 1.53	27.83 ± 11.26
PA02E	121.33 ± 11.59	12 ± 0.05	49.33 ± 2.08	60.33 ± 4.51	38 ± 5.11	2 ± 0.00	20.00 ± 1.00	1.13 ± 0.648	301 ± 115	1551.5 ± 308.18	107 ± 0.58	28.16 ± 6.56
PA02B	100.33 ± 8.50	15 ± 1.000	38 ± 2.00	52.67 ± 5.77	54.67 ± 4.73	3.66 ± 0.57	21.67 ± 0.58	1.36 ± 0.06	445 ± 212	1483.8 ± 381.3	106 ± 3.21	25.01 ± 2.62
PA03C	132 ± 5.20	14 ± 1.00	47.33 ± 0.58	54 ± 4.58	55.67 ± 5.86	2 ± 1.00	19.67 ± 1.53	1.74 ± 0.786	455.3 ± 137	3955 ± 558.79	107 ± 0.80	31.23 ± 13.08
Faisalabad-08	104.3 ± 21.4	12.667 ± 0.58	35 ± 1.73	49.33 ± 7.51	61.33 ± 6.43	2.66 ± 1.15	21.00 ± 2.00	2.65 ± 0.472	125 ± 78.9	1715.8 ± 366.13	107 ± 0.50	43.03 ± 3.44
NARC 2009	91.67 ± 3.79	12.667 ± 1.53	36.33 ± 2.08	40 ± 2.00	60.33 ± 2.52	1.33 ± 0.57	19.00 ± 1.00	2.98 ± 0.775	583 ± 183	4712.5 ± 0.00	108 ± 5.03	34.94 ± 3.21
NARC 2011	91.33 ± 5.69	12 ± 1.00	38 ± 2.00	39.33 ± 3.51	58.33 ± 2.52	1.33 ± 0.57	20.67 ± 0.58	3.01 ± 0.078	457 ± 19.2	4379 ± 41.01	107 ± 4.04	28.83 ± 5.24
Sehar-06	88.67 ± 4.04	12.33 ± 2.52	31 ± 2.00	39 ± 4.36	59.67 ± 8.74	1 ± 0.00	20.67 ± 1.53	2.07 ± 0.137	391 ± 390	1836.7 ± 343.3	107 ± 0.60	27.56 ± 3.17
PA03F	121 ± 7.00	13.33 ± 1.53	48.33 ± 1.16	46.33 ± 7.51	42 ± 7.94	3.66 ± 0.57	21.00 ± 1.00	1.18 ± 0.03	346 ± 222	1352 ± 66.6	111 ± 0.70	35.4 ± 7.65
PA07A	85.33 ± 15.57	12 ± 1.00	32 ± 1.73	36.67 ± 4.51	47.67 ± 2.08	1.66 ± 0.57	20.33 ± 2.08	1.31 ± 0.132	422 ± 284	1984 ± 168.5	101 ± 0.50	33.5 ± 3.87
PA02J	97 ± 6.24	12.667 ± 1.53	34.33 ± 2.89	47.33 ± 6.51	48.33 ± 1.53	1 ± 0.00	19.33 ± 2.08	1.72 ± 0.399	629 ± 39.8	2657 ± 312.7	105 ± 1.00	36.75 ± 5.77
PA06I	92.33 ± 4.73	13.333 ± 0.58	36.33 ± 2.51	48 ± 4.58	54.33 ± 1.53	2.66 ± 0.57	21.00 ± 1.00	1.82 ± 0.16	201 ± 57	1493.5 ± 394.7	103 ± 2.08	31.45 ± 15.29
PA03E	131.33 ± 6.03	14.333 ± 0.58	52.67 ± 2.89	50.33 ± 8.50	48.67 ± 3.79	1.66 ± 0.57	21.00 ± 1.00	1.79 ± 0.283	175.3 ± 33.9	2417.8 ± 333.2	107.67 ± 0.58	19.89 ± 13.74
PA06A	96.67 ± 3.06	12 ± 1.00	34 ± 1.00	51 ± 1.00	54.6 ± 6.07	3.33 ± 1.52	19.00 ± 3.00	1.62 ± 0.502	135.3 ± 52.69	2443.25 ± 196	104 ± 2.31	33.04 ± 13.3
PA06F	87.333 ± 1.53	10.67 ± 1.15	30.33 ± 1.16	39.67 ± 4.04	39.67 ± 6.2	0.66 ± 0.57	20.00 ± 1.00	0.91 ± 0.824	209.6 ± 32.7	2066.5 ± 379	101 ± 0.5	49.37 ± 10.7
PA02A	115.33 ± 6.11	11.333 ± 1.53	41.33 ± 0.58	50.33 ± 1.53	43.67 ± 4.04	1.66 ± 0.57	21.33 ± 2.89	1.45 ± 0.647	156.6 ± 36.85	3113.8 ± 938	104 ± 2.65	51.74 ± 3.26

PH = Plant height (cm); SL = Spike length (cm); PL = Peduncle length (cm); LA = Leaf area (cm^2); NGPS = Number of grains per spike; NIS = Number of infertile spikelets; The values after ± sign indicate standard deviation; NFS = Number of fertile spikelets; GWPS = Grain weight per spike (g); NFT = Number of fertile tillers; GY = Grain yield (kg/ha); SI = Spike initiation (days); 1000 GW = 1000 grain weight (g); The values after ± sign indicate standard deviation

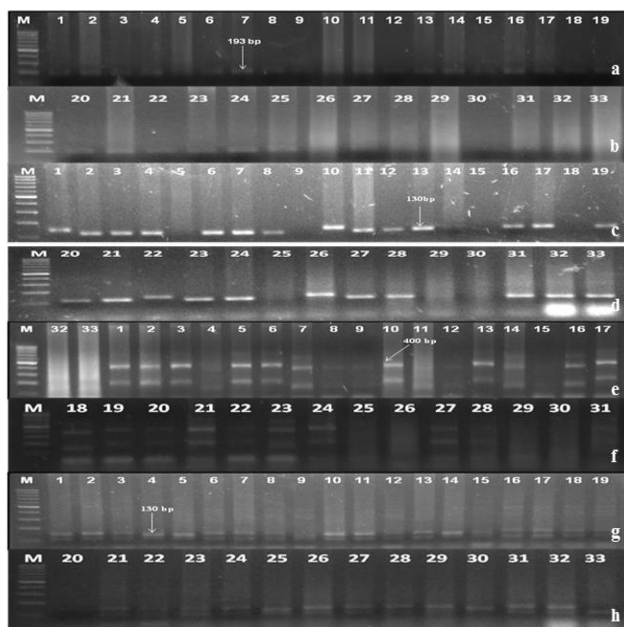

Fig. 1. PCR amplification of different markers (a&b) Xgwm159 locus (193 bp) linked to K^+ accumulation trait under high salt stress in advanced wheat lines. No. 1-33 denotes advanced wheat lines with four check varieties (c&d) Xgwm533 locus (130 bp) in advanced wheat lines showing K+ accumulation under salt stress (200 mM) with 100bp marker (e&f) Xgwm174 loci (400 bp, 350 bp, 200 bp and 150 bp) linked to trait of biomass production and grain yield under salt stress (100 bp marker) (g&h) Xgwm121 linked to trait of spikelets sterility on the 7A chromosome with the 130 bp and 126 bp loci (100 bp marker)

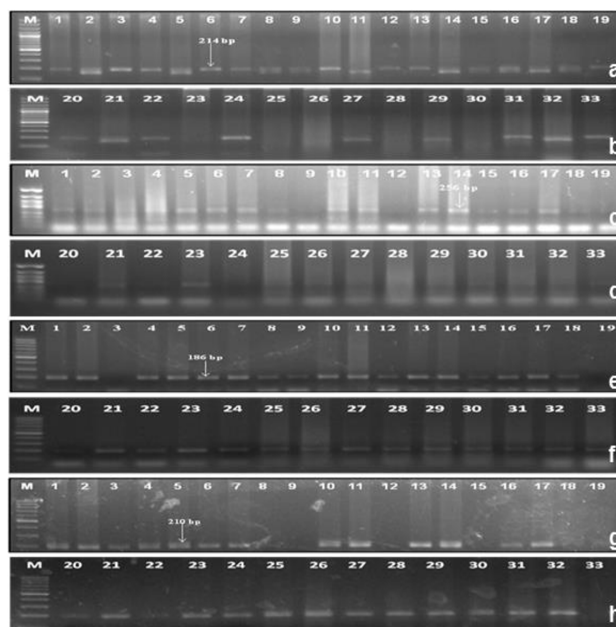

Fig. 2. PCR amplification of different markers (a&b) Xcfd49 marker for salt tolerance trait linked to sterile spikelets per spike on locus (214bp) with 100bp marker (c&d) Xgwm2 marker linked to grain weight per main spike in twenty nine advanced wheat lines and four check varieties (Marker = 50 bp) (e&f) Xgwm18 marker for salt tolerance trait K accumulations on locus (186 bp) in thirty three advanced wheat lines including the check varieties (Marker = 100 bp) (g&h) Xgwm332 on locus (210 bp and 189bp) trait linked to sterile spikletes per spike (Marker = 100 bp

In the present study, SSR marker Xcfd49 amplified 214 bp and 150 bp fragments for the presence of salt tolerance trait. In 29 advanced wheat lines, PA06G, PA06J, PA02H, PA06C, PA06E, PA06B, PA02F, PA03D, PA06H, PA03C and NARC-2009 (check variety) amplified both the fragments. Sehar-06, PA07A, PA02J and PA06F genotypes did not amplify any allele (Fig. 2 a&b). This marker is linked with the trait of sterile spikelets per spike on the chromosome 6D (Shahzad, 2007). Similarly, in a previous research study by Ahmad *et al*., (2013) most of the genotypes showed variations for the two allele that was in range of 150-214 bp. Microsatellite marker Xgwm2 was used to amplify the salt tolerance trait in 33 advanced wheat lines. All the genotypes including four check cultivars amplified the expected band fragment and PA03F did not amplify any band whereas the check varieties showed positive bands for the target trait (Fig. 2 c&d). Xgwm2 is linked to the trait of grain weight per main spike mapped at chromosome 3D, 3AS and 2AS with expected band size of 256bp (Roeder *et al*., 1995; Ahmad, 2011). Xgwm18 amplified targ*et al*leles in 31 lines including the 4 control varieties with the expected fragment size of 186 bp. Only two lines PA07B and PA02E were found to be negative and the remaining all the genotypes gave positive result for salt tolerance trait (Fig. 2 e&f). PA06D, PA06J, PA02K, PA02I, PA03A, PA06E, PA03D, PA03C, Sehar-06 (Check variety), PA07A, PA02J and PA06F showed positive result for both the allele and PA06B, PA02G and PA06C were failed to amplify any fragment for DNA marker Xgwm332 (Fig. 2 g&h). Similar to our findings, Imtiaz *et al*., (2001) and Somers *et al*., (2004) also reported that DNA marker Xgwm332 amplified fragments of 215 bp (stripe rust) and 189 bp for the salt tolerance trait.

All the genotypes showed 100% result for the presence of target trait (Fig. 3 a&b). It was concluded from the above result that the presence of this gene was responsible for the uptake of Na^+ ions concentration in salt condition. High concentration of Na^+ not only disturbed the leaf tissues but also affected the K^+ uptake, therefore K/Na^+ ions concentration was reduced. Due to this high concentration, the efficiency of sterile spikelets is increased (Mahar *et al*., 2003; Munns, 2007). Microsatellite marker Xgwm371 is linked to the trait of biomass per plant and Na^+ ion concentration at 100 mM salt stress on the chromosome 5B (Shahzad, 2007). This marker was used to detect the presence of this trait in 33 lines. The expected fragment size of 200 bp was not amplified in PA02F, PA02B, NARC-2009 and NARC-2011. Faisalabad-08, Sehar-06 and remaining advanced wheat lines amplified the fragment (Fig. 3 c&d). Two wheat lines PA06B and PA02B were negative for Xgwm539, while the remaining 31 lines were positive for salt tolerance trait with amplicon size of 150 bp (Fig. 3 e&f). Among 29 genotypes, PA07B, PA03A, PA06A and PA02A were negative for Xgwm604, while the remaining 25 lines including Faisalabad-08 and Sehar-06 (check variety) were detected with positive allele (Fig. 3 g&h). According to Shahzad (2007), salt tolerance trait (height of wheat plant under salt stress) was linked with Xgwm604 marker on chromosome 5B. SSR marker Xgwm645 was linked to the trait of grains per spike and the Na^+ ion concentration at 100 mM present on the chromosome 3D (Roder *et al*., 1998). In the present study, twenty-nine advanced wheat lines were used to detect the salt tolerance trait, 5 genotypes PA02B, PA03F, PA02J, PA06A, and PA02A did not amplify the targ*et al*lele and most of the lines including NARC 2011 (Check variety) showed positive result (Fig. 3 i&j).

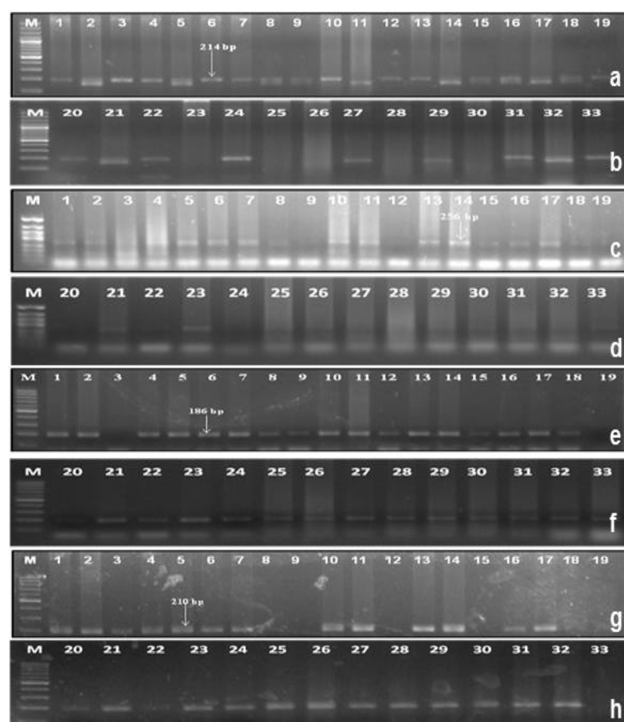

Fig. 3. PCR amplification of different markers (a&b) Xcfd49 marker for salt tolerance trait linked to sterile spikelets per spike on locus (214bp) with 100bp marker (c&d) Xgwm2 marker linked to grain weight per main spike in twenty nine advanced wheat lines and four check varieties (Marker = 50 bp) (e&f) Xgwm18 marker for salt tolerance trait K accumulations on locus (186 bp) in thirty three advanced wheat lines including the check varieties (Marker = 100 bp) (g&h) Xgwm332 on locus (210 bp and 189bp) trait linked to sterile spikletes per spike (Marker=100 bp

Microsatellite marker Xgwm674 was used to evaluate the 33 advanced wheat lines linked to the trait of K/Na^+ at 200 mM salt stress on the location of chromosome 3A (Shahzad, 2007). All lines including check variety showed positive result for both allele fragments 140 bp and 130 bp except some genotypes i.e., PA02D, PA03B, PA02H, PA06C, PA06E, PA06B, PA02E and PA02B that showed negative result for 140 bp fragment (Fig. 4 a&b). DNA marker Xgwm11 was linked to the trait of K/Na concentration at 100 mM on chromosome 1B with a single allele product size of 203 bp (Somers *et al.*, 2004). All the genotypes including the check varieties showed presence of salt tolerance trait except for 7 genotypes PA07B, PA06D, PA06C, PA06B, PA02F, PA06A and PA06F that were negative for salt tolerance (Fig. 4 c&d).Xgwm133 marker is present on the chromosome 4DL which is linked to the trait of plant height, grain weight per main spike, K/Na at 100mM and spikes per plant (Somers *et al.*, 2004). All the advanced wheat lines including 4 check varieties showed the fragment size but the genotypes PA02C, PA07B, PA07A and PA02J lines were failed to show the presence of this fragment size (175 bp) (Fig. 4 e&f). Similar studies were conducted by the Gorham *et al.*, (1997) and Dubscovsky *et al.*, (1996) that *Kna1* gene was responsible for the K^+/Na^+ discrimination on 4DL chromosome of wheat genome and these traits are controlled quantitatively. Xgwm205 marker amplified the product size of 137 bp for salt tolerance trait in all 33 genotypes (Fig. 4 g&h). Quarrie *et al.*, (2005) found that the Na^+ elimination and K^+ accumulations are controlled by genome A and in mostly chromosome 5A. Nax2 leaf present on the 5A chromosome is responsible for the reduced rate of Na^+ from root to shoot and accumulation of K^+, thus resulting in improved K^+ versus Na^+ intolerance (known as K^+/Na^+ ratio) (James *et al.*, 2006). Three genotypes PA06A, PA06F and PA02A did not amplify the target locus of Xgwm335. The remaining 30 advanced wheat lines *showed a positive result (amplified product 200 bp) (Fig. 4 i&j). It has been documented that QTL mapped for K^+/Na^+ ratio at 100 mM and 200 mM salt stress on chromosome 5B contributed positively for the trait of salt stress. Reduction of Na^+ concentration and improved K^+ uptake are the important indicators of salinity tolerance in cereal crops plants (Marschner, 1995; Hu & Schmidhalter, 1997). It is ability of plants to reduce Na^+ transport into shoot for the maintenance of growth mechanism and protection of the metabolic activities in growing and dividing cells from the toxic effect of Na^+ salt stress (Razmjoo *et al.*, 2008).

Xgwm383 is linked to the hundred grain yield and harvest index trait in plant genome with the amplified locus of 157 bp at chromosomes 3B (Gandhi, 2005). All the lines including control varieties showed presence of salt tolerance trait except for PA07A, PA06G, PA02K and PA03A lines that showed susceptibility to the salt tolerance (Fig. 5 a&b). Results in this study are contradictory to the findings of Singh & Sharma (1994), which might be due to the differences in genetic material and environment. Only two QTL for percent water were identified. The first QTL was present on the long arm of chromosome 3D in the interval GWM383– P68M86-6 whereas the QTL for grain weight was also identified by Amin *et al.*, (2013). Amongst the 21 chromosomes, chromosome 3B with total of 27 QTL and 2 distinguishing cluster regions were the most significant (Azadi *et al.*, 2014). Xgwm111 marker linked to Na^+ concentration at 200 mM salt stress on the chromosome location of 7D (Amin, 2002). The product size of targ *et al* lele is 150 bp and all lines showed presence of expected fragment size for salt tolerance trait (Fig. 5 c&d). The dominant marker cfd19 is linked to the trait of grains per spike (Amin, 2002). During the present study, most of the genotypes could not amplify 267 bp fragment band. Four check varieties in which Faisalabad-08 and Sehar-06 amplified the 294 bp fragment (Fig. 5 e&f). Marker psp3113 was linked to the trait of Na^+ ion at 100 mM, K/Na^+ at 100 mM and K/Na^+ at 200 mM salt stress on the chromosome 7D (Amin, 2002). All the twenty-nine lines showed presence of that trait and amplified the fragment of 162 bp (Fig. 5 g&h). All the result showed polymorphism and Faisalabad-2008, Sehar-2006 and NARC-2009 amplified the 189 bp fragment for DNA marker Xgwm148. Some lines PA07B, PA02E, PA07A, PA02J, PA06I, PA03E and PA06A did not amplify any fragment (Fig. 5 i&j). In a previous research study by Ahmad *et al.*, (2013), DNA marker Xgwm148 was used to evaluate the advanced wheat lines with expected band fragments of 189bp linked to K^+ accumulation at 200 mM at the chromosome location of 2B.

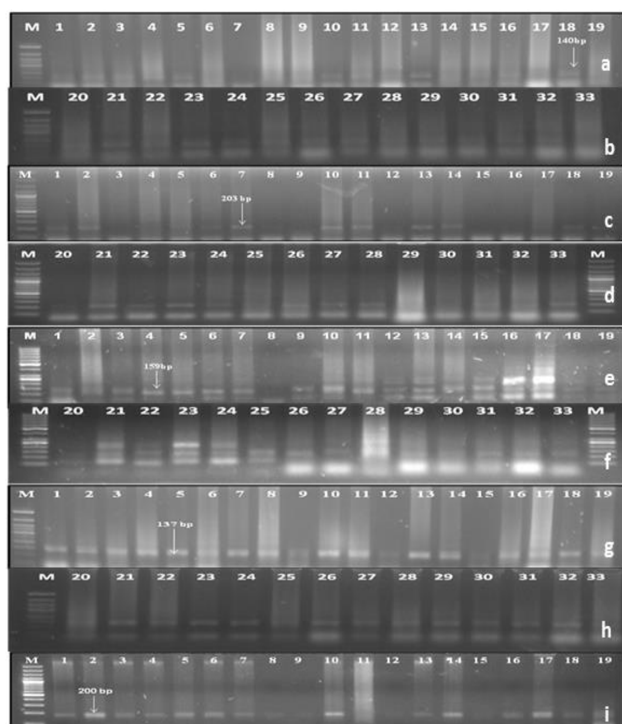

Fig. 4. PCR amplification of different markers (a&b) Xgwm674 marker to detect the salt tolerant trait linked toK/Na$^+$ at 200mM concentration in 33 advanced wheat lines including four check varieties (M=50 bp) (c&d) Amplification of thirty three genotypes including four check varieties using Xgwm11 marker for salt tolerance trait linked toK/Na concentration at 100 mM at locus 203 bp (M=100 bp) (e&f) PCR product of twenty nine advanced wheat lines with four check varieties by marker Xgwm133 linked toplant height, grain weight per main spike, K/Na$^+$ at 100mM and spike per plant (M=50 bp) (g&h) PCR amplification patterns of twenty nine advanced wheat lines with four check varieties using marker Xgwm205 linked to grain yield per main spike (M = 50 bp) (i) Microsatellite of thirty three genotypes including four check varieties at loci Xgwm335 linked toK/Na$^+$ ions concentration at 100 mM and also at 200 mM on the chromosome 5B (M = 100 bp).

Evaluation of morphological characteristics of advanced wheat lines: Advanced wheat lines showed significant differences (p<0.001) for plant height (Table 4). PA03B exhibited the maximum height (141 cm) and PA07B gave the minimum (80.67 cm) plant height compared to the check varieties such as NARC 2009, NARC 2011, Faisalabad-08 and Sehar-06. Mean data showed that PA02B had the longest spike (15 cm) and PA06C produced the shortest spike (10 cm). The highest peduncle length was observed in PA03A and PA03E (54 and 52.67 cm) but the 4 check varieties had the shortest peduncle length in the range of 31 to 38 cm. Advanced wheat lines PA02E and PA06D had the largest leaf area (60.33 cm^2 and 59.00 cm^2), respectively and PA07A (36.67 cm^2) line showed the shortest leaf area with regards to the check varieties (Table 4). Highly significant difference (p≤0.001) was observed among the genotypes for number of grains spike^{-1}. Twenty nine advanced wheat lines and 4 check varieties, PA02K had the maximum number of grains spike^{-1}. Correlation of number of grains spike^{-1} with number of fertile spikelets/spike, grain yield per spike, grain yield and number of infertile spikelets/ spike, 1000 grain weight were negative and non-significant (Table 4).

PA06F line showed the least number of infertile spikelets spike^{-1} (0.66) and the maximum number of infertile spikelets spike^{-1} was observed in PA02B (3.66) (Table 4). Significantly positive correlation of number of infertile spikelets spike^{-1} with days to spike initiation was observed and significantly negative correlation was found with grain yield (Table 4). Mean data showed that most of the lines did not exhibit variations in case of number of fertile spikelet per spike as compared to the control varieties. Data revealed highly significant differences (p≤0.001) among the 29 advanced wheat lines for grain weight spike^{-1}. Mean data showed that genotypes PA06B, PA06D and PA06F produced lesser yields (0.79, 0.917 and 0.907 g), respectively as shown in Table 4. The highest yield was recorded in the remaining advanced wheat lines as well as control varieties.

The highest number of fertile tillers was found in PA06B (646) which indicated the highest grain yield compared to other advanced wheat lines. Significantly positive correlation was observed with grain yield but significantly negative correlation was found with the days to spike initiation and 1000 grain weight (Table 4). Significant difference (p≤0.05) was observed among the 29 genotype for grain yield. Mean data showed that PA06B (5310.6 kg/ha) had the highest potential to produce high grain yield per line and the lowest yield was calculated in PA06D (1205.9 kg/ha) compared to the four control varieties. Mean data showed that control varieties were initiated at the 107 days after sowing and higher number of days required to spike initiation were observed in PA03F, PA07B and PA03A advanced wheat lines (111days, 110days, 111days) and the lesser days to spike initiation were observed in the PA06D (102), PA02H (103), PA06C (101), PA02K (103), PA06E (101), PA06B (101), PA06H (104), PA07A (101), PA06I (103), PA06A (104) and PA06F (101) genotypes (Table 4).

The correlation study revealed that grain yield (kg/ha) was significantly positive correlated with grain yield per spike, number of fertile tillers and number of grains per spike but showed significantly negative correlation with number of infertile spikelet's per spike and 1000 grain weight (Table 5). Mondal *et al*., (1997) reported significantly positive correlation of tillers per plant and ear length with yield per plant whereas Raut *et al*., (1995) also demonstrated the number of grains per spike and number of spikelets per spike by 1000 grain weight significantly positive correlated with spike length and showed negatively significant correlation with grain yield per spike, grain yield and number of fertile tillers. Sinha *et al*., (2006) reported that 1000 grain weight had positive and non-significant correlation with yield per plant under irrigated timely sown wheat. Leaf area had significantly positive correlation with plant height, peduncle length, spike length and number of infertile spikelets per spike.

Table 5. Correlation coefficient among all the morphological parameters of 33 advanced wheat lines including four check varieties during 2013-2014.

	PH	SL	PL	LA	NGPS	NISPS	NFSPS	GYPS	NFT	GY	DSI
SL	0.1593										
PL	0.802*	0.2254*									
LA	0.4515*	0.3074*	0.4132*								
NGPS	-0.1522	0.0578	-0.2223*	-0.2043*							
NISPS	0.0104	0.1219	0.0236	0.1317	-0.0988						
NFSPS	-0.0797	0.2921*	-0.0862	0.0898	0.2054*	-0.0196					
GYPS	0.034	0.0165	0.1183	-0.1311	0.6325*	-0.0997	0.1264				
NFT	0.0937	-0.0558	0.0019	-0.1357	0.0823	-0.1207	-0.1523	0.0416			
GY	0.1802	-0.1818	0.1386	-0.0817	0.2364*	-0.2352*	-0.1121	0.4812*	0.3842*		
DPI	0.1637	0.3262*	0.2733*	0.1507	0.0957	0.2781*	-0.0497	0.2438*	-0.2272*	-0.0528	
1000GW	-0.1272	0.202*	-0.087	0.0285	-0.1296	0.1629	0.0763	-0.3598*	-0.3135*	-0.823*	0.0788

PH = Plant height (cm); SL = Spike length (cm); PL = Peduncle length (cm); LA = Leaf area (cm^2); NGPS = Number of grains/ spike (g); NISPS = No. of infertile spikelets/spike; NFSPS = Number of fertile spikelets/spike; GYPS = Grain yield per spike (g); NFT = Number of fertile tillers; GY = Grain yield (kg/acre); DSI = Days to spike initiation; * = Significant at $p\leq 0.05$.

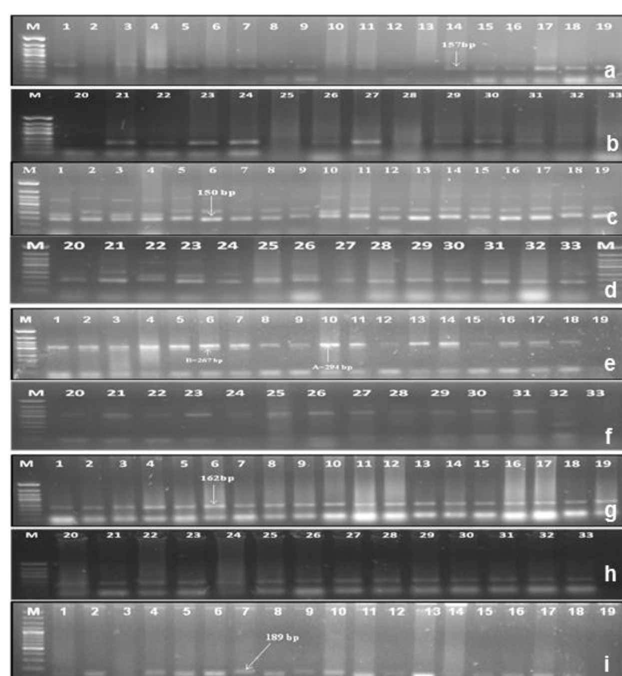

Fig. 5. PCR amplification of different markers (a&b) Amplification of twenty nine genotypes with four check varieties using Xgwm383 for evaluation of salt tolerance trait hundred grain yield and harvest index at locus 157 bp (M = 50 bp) (c&d) Electropherogram of 33 advanced wheat lines including four check varieties with Xgwm111 marker with the trait linked to Na concentration at 200 mM at locus 200 bp and 150 bp (M = 50 bp) (e&f) PCR products of marker Xcfd19 linked to grains per spike, Na^+ PCR amplification of twenty nine advanced wheat lines with four check varieties using marker psp3113 on 2.5% agarose gel (M = 50 bp) (i&j) Xwm148 marker for evaluation of thirty three genotypes including four check varieties on locus (189 bp), trait linked to K^+ accumulation at 200 mM (M = 100 bp)

Cluster analysis of advanced wheat lines: Dendogram was constructed on the basis of coefficient of similarity matrix. Coefficient of similarity ranged from 79% to 100% indicating the divergence between all the advanced wheat lines (Fig. 6). Cluster analysis divided the 33 lines into 6 groups. Group 1 included nine advanced wheat lines with one check cultivar (Faisalabad-08). Group 2 consisted of two lines (PAO6I and PAO3E) and group 3 included the 12 lines with three check varieties (NARC-2009, NARC-2011 and Sehar-06). Group 4 contain two lines (PAO6B and PAO6C), group 5 also consists of two genotypes (PAO3F and PAO2A) and group 6 included three advanced wheat lines (PAO2J, PAO6F and PAO6A). Variety pair with higher value of coefficient is more diverge than pair with lower value.

Group 1 consisted of nine advanced wheat lines. PAO2C and PAO3A had the 93% genetic similarity index. PAO6G showed divergence from above two genotypes. In this group, PAO6J and PAO3D had the greatest genetic similarity at 99% and PAO3C showed divergence from it. PAO2K, PAO2D and Faisalabad-08 (control) had some divergence to each other from above result but all these lines were present in one group showing that it had some genetic similarities to the check varieties. Group 2 comprised of two lines with PAO6I and PAO3E that showed close genetic similarity of 98% to each other. Group 1 and group 2 were present at the same clades that represented similarity to each other. Twelve lines including three varieties were present at the group 3. PAO6D and PAO2I were 96% similar at genetic level but PAO7A showed deviation from the previous lines. PAO2G and NARC-2011 had 100% similarity to each other and these three verities such as NARC-2009, NARC-2011 and Sehar-06 is present at the same clad. These two varieties (PAO2G and NARC-2011) showed their common origin by indicating no genetic distance value (0.00) between them. All the lines of group 3 were closely related to control varieties.

Coefficient of similarity (96% and 94.5%) was present between PAO2F and PAO6H; PAO3B and PAO6E present at the same group 3 on the same clad respectively (Fig. 6). PAO2H showed divergence from above 4 advanced wheat lines but present at the same clad. PAO7B placed a unique isolated place in dendrogram. Group 1, 2 and group 3 had the common

genetic clad and they showed some similarity to each other. Group 5 comprised of only two lines. PAO6B and PAO6C had a 0.93 coefficient of similarity and presented some similarity with the previous group due to common clad. Group 6 consisted of three lines with PAO2J, PAO6F and PAO6A. These three genotypes shared some similarity because they had a common clad comprising of coefficient of similarity in range of 84 to 88%. PAO2E, PAO2B and PAO7B were not included in any group (Fig. 6).

PAO6F and PAO2J closely resembled with coefficient of genetic similarity at (88%) but PAO6A showed divergence with 84%. To study relationship in 33 advanced wheat lines, coefficient of similarity varied from 79 to 100%. The maximum genetic similarity 100% was recorded between PAO2G and NARC-2011 followed by 99% similarity between PAO6J and PAO3D. The minimum genetic similarity (86%) was computed between PAO3F and PAO2A followed by PAO2J and PAO6F (88%).

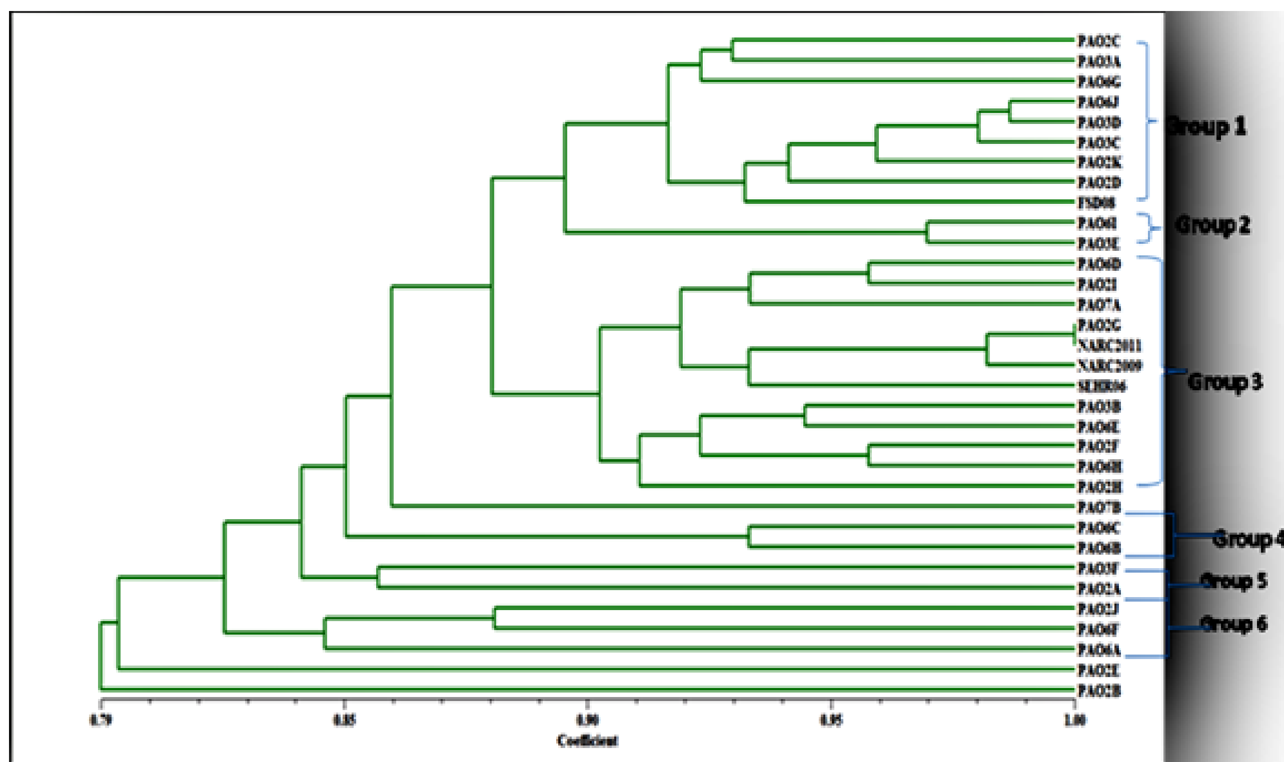

Fig. 6. Genetic relationships between 33 advanced wheat lines including four control varieties generated by SSR data shown by UPGMA cluster analysis based.

Conclusion

Advanced wheat lines PA06B, PA02K, PA03B, PA02G and PA03C were found to be the most salt tolerant and therefore could be used as new sources of salt tolerance. Significantly dominant possessions for yield and yield related traits in salt tolerant wheat lines suggest that selection for yield under salinity stress could be effective. DNA marker gwm2, gwm335, gwm383, psp3113, cfd19, gwm371, gwm539, gwm604, gwm645, gwm111, gwm133, gwm205, gwm121, gwm159, gwm18 and gwm357 showed presence of expected fragments linked to salt tolerance traits in salt tolerant wheat lines. These markers would be used to evaluate wheat genotypes for salinity tolerance.

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