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Original article

Genetic diversity in exotic oat germplasm & resistance against barley yellow dwarf virus

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ABSTRACT

Oat (*Avena sativa* L.) is an important fodder crop of Pakistan, though with low productivity. The present study was conducted to evaluate the performance and genetic diversity of exotic oat germplasm, with emphasis on cereal yellow dwarf virus resistance. A total of 16 exotic line (introduced from Aarhus University Denmark) and 1 local line (provided by The University of Agriculture Peshawar), were grown during the season 2017–18 in Completely Randomized Block Design with three replications across two locations of Khyber Pakhtunkhwa i.e., Peshawar and Kohat. Field testing enabled to collect the data on BYDV incidence, BYDV severity, aphid infestation, plant height, leaf area, panicle length, panicle weight, spikelets per panicle, 1000 grain weight (g), grain yield (g), biological yield (g) and harvest index (%). Prevalence of BYDV was variable across location and over time. Six weeks data showed high disease pressure at Peshawar (85%), with SA-O-01 genotype having AUDPC value of 95%. Almost all the varieties showed less tolerance towards the Aphids attack. Line SA-O-15 showed the maximum 1000 grain weight (42.6 g) at Kohat, while SA-O-4 showed the maximum 1000 grain weight (60.7 g) at Peshawar. Line SA-O-05 (3634 g per (0.9 m^2) plot) gave the maximum biological yield at Kohat station, while Line SA-O-01 gave the maximum biological yield (2517 g) at Peshawar. Mean grain yield for Kohat was recorded 0.155 g per (0.9 m^2) plot while for Peshawar it was 0.231 g per (0.9 m^2) plot. At Kohat line SA-O-10 produced the maximum grain yield (0.229 g), while line SA-O-12 produced the maximum grain yield at Peshawar (0.288 g). Molecular genotyping with a set of 4 RAPD primers revealed substantial diversity among17 oat lines. A total of 23 loci were amplified showing a high level of variations and polymorphism among the proposed lines. The maximum number of loci was recorded for GLA-04 (8), while the minimum number of loci was recorded for GLD-18 (4). Among the tested RAPD primers the maximum gene diversity (0.529) was recorded for loci GLA-03B230, GLA-04B130, GLA-04B300, GLB-05B150 and GLA-18B100 while the minimum (0.118) genetic diversity was recorded for loci GLA-03B600, GLB-05B330 and GLA-18B500. A clear divergence was found between most of the exotic oat lines. The observed genetic diversity in exotic oat germplasm and its resistance towards Barley Yellow Dwarf virus could be useful for oat genetic improvement and broadening the genetic background of cultivated oat germplasm.

1. Introduction

Oat (*Avena sativa L.*) is an important member of the family of Gramineae, originated in Asia and modern oat has been produced from Asian wild oat, which is mainly considered as grass (Coffman, 1977). It is mainly used as a fodder crop, but in many countries, it is also used as a food crop, such as in Scotland, Germany and Scandinavia (Welch, 2012). Oat gives sustainable production even in marginal environment including cool wet climate and low soil fertility (Hoffman, 1995). The major growing areas

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of Oat lies between 40° and 60°N latitudes in Asia, Europe and America (Forsberg and Reeves, 1992).

Oat ranked sixth in the world cereal production after wheat, maize, rice, barley and sorghum (Boczkowska and Tarczyk, 2013). The high fibrous and enhanced nutritional value make it a healthy dietary supplement for a long period of time (Rasane et al., 2015). Oat also contains various bioactive phytochemicals e.g., vitamin E, phenolic acid and flavonoids (Peterson, 2001). Two important biologically active phytochemicals are also produced by Oats viz. Avanthramides and saponins. Consumption of Oat enriched with Avanthramides extract increases antioxidant potential in healthy humans (Chen et al., 2007). Oat consumption also reduced the risk of coronary heart diseases by inducing the ROS (Reactive Oxygen Specie) (Berg et al., 2003) and also help in improvement of symptoms related to obesity and diabetes (Li et al., 2016). With the medicinal use of this crop, oat are also produced in whiskey, coffee options, cosmetics, fiber paper, pillow filling etc.

In Pakistan, oat utilization is mostly limited to feed animals, because of its softness compared to other fodder. Nutritionally, this fodder crop is very rich and fulfills the nutritional requirements of animals such as Total Digestible Nutrients (TDN), digestible raw proteins, fats, vitamins and minerals such as phosphorus and iron (Sterna et al., 2016). Similarly, oats are the favorite food for horses, dairy cows and poultry (Menon et al., 2016). Despite the importance of the crop, its production in Pakistan is very limited. Low production and yield is due to limited efforts for the genetic improvement of oats in Pakistan, particularly to crop limiting factors which are adversely affecting biological development and crop production (Khalil, 2008). In various biotic factors imparting negative impact on oats are rust, powdery mildew, barley/Cereal yellow dwarf virus (B/BYDV), scab and ergot. Cereal Yellow Dwarf virus is an important disease among all the rusts, which is often prevalent under the humid and cold weather (Simons, 1985) in the oat crop.

In 1951, the characteristics of Barley Yellow Dwarf Virus (BYDV) were first seen in barley in California, giving its name of BYDV (Oswald and Houston, 1951). Later, the disease was detected in Oat along with wheat (*Triticum eastivum*), thus giving its general name Cereal Yellow Dwarf Virus (CYDV). The disease remained documented all over the world. In Pakistan, the disease was initially reported in 1964, near the Pakistan-Afghan border in the wheat crop (Aslam and Ahmed, 1987). CYDV is characterized by stunted growth, leaf fall and discoloration (red or purple color beginning from leaf tip and moving towards leaf base). The intensity of high light and cold temperatures (15–18 °C) was found to favor the expression of BYDV symptoms (D'Arcy and Burnett, 1995).

Aphids are considered as a vector for the transmission of BYDV at short and long distances. Various studies have been conducted which mentioned the involvement of around 25 aphid species in BYDV transmission (Halbert and Voegtlin, 1995). Aphids transmits the virus by removing the phloem sap from infected plants and then feeding on other plants, thus sending viruses to healthy hosts (Smith et al., 1968). The amount of injury depends on the size of the infestation, time of infection during the growing season, and the efficiency of the virus transmission (Wadley, 1929). The weather plays a vital role in the Aphids multiplication and movement as these can be transported hundreds of miles through air. The degree of aphid infestation could vary as per host genetic make-up and the level of diversity. Little, however, is known regarding variability in Pakistani oat germplasm in terms of BYDV infection and aphid infestation, leading to reduction in oat yield. Improved oat lines must be developed in diverse genetic stocks with special emphasis on disease resistance, particularly to BYDV.

Genetic diversity for the survival adaptation of crops to certain agronomic condition leads to high yield and desirable characters in the crop. Characterizing any germplasm with morphological characters could be easy and cheap but linkage of these traits with phenotypic selection is week and thus very few of them could be utilized (Islam and Shepherd, 1992). Development in the molecular genetic techniques for the characterization of crop/weed species, using restriction fragment length polymorphism (RFLP) (Paterson et al., 1991; Ulloa et al., 2002), simple sequence repeats (Röder et al., 1995), and randomly amplified polymorphic DNA (Williams et al., 1990) had converted the visions of employing molecular biology to molecular breeding. Molecular markers are the effective tool for characterization and evaluation of genetic resources providing information both within and among the specie (Boczkowska and Tarczyk, 2013). Using RAPD markers, various genotypes of crops/weeds were characterized (Mitra et al., 1998) because they require small quantity of genomic DNA. It is a fast and easy way for generating genotypic data (Nissen et al., 1995). SSR markers are also commonly used markers but in plant genome distribution of SSRs are far more common (Cardle et al., 2000).

Considering the economic importance and health benefits of oats and the BYDV and aphids impact on production and yield, the exotic and indigenous germplasm should be tested for their variability, particularly in terms of BYDV and aphid infestation. It is essential to identify, deploy and characterized resistance in the oat germplasm. This current study will assess the diversity of exotic oat germplasm for morphological and yield parameters, across multi-locations in Pakistan using RAPDs molecular marker and also assess the prevalence of BYDV disease in oat germplasm at Peshawar and Kohat.

The present study was designed with the following objectives (i). To assess the diversity in exotic oat germplasm across two locations (Peshawar & Kohat) for morphological and yield parameters; (ii). to assess the prevalence of BYDV in oat germplasm across Kohat & Peshawar and the weekly progress of disease at Peshawar; (iii). to assess the genetic diversity in exotic oat germplasm and assess the potential relationship with disease infestation.

2. Materials and methods

The study was carried out to assess the morphological and yield related diversity in oat germplasm introduced from Europe at the Institute of Biotechnology & Genetic Engineering, the University of Agriculture, and Peshawar, Pakistan. Special emphasis was given to the status of BYDV infestation and level of BYDV resistance. The experiment was a part of on-going cereal genetic improvement programme of the institute.

2.1. Selection of genotypes and locations

Seeds of 16 exotic oat genotypes from Europe and one local check variety from Agriculture university Peshawar Pakistan were sown at two locations viz. Peshawar (at IBGE Research Farm, the University of Agriculture, Peshawar), and Kohat (Barani Agriculture Research Station Kohat) during oat growing Season 2017–2018 (Table 1).

2.2. Field layout, sowing and crop husbandry

The selected oat lines were tested at the selected locations using a randomized complete block design (RCBD) with three replications. Every plot within each replication was consisted of two rows of 1.5 m length with row-to-row distance of 0.3 m. Crop production strategy at each location was followed according to crop recommendations for respective locations.

Table 1
The set of 16 exotic oat lines and 1 local check line selected for testing their barley yellow dwarf virus through multilocation testing and molecular markers.

S. No.	Genotype	Detail	S. No.	Genotype	Detail
1	O-SA-1	Introduced line	10	O-SA-10	Introduced line
2	O-SA-2	Introduced line	11	O-SA-11	Introduced line
3	O-SA-3	Introduced line	12	O-SA-12	Introduced line
4	O-SA-4	Introduced line	13	O-SA-13	Introduced line
5	O-SA-5	Introduced line	14	O-SA-14	Introduced line
6	O-SA-6	Introduced line	15	O-SA-15	Introduced line
7	O-SA-7	Introduced line	16	O-SA-16	Introduced line
8	O-SA-8	Introduced line	17	O-SA-17	Local control line
9	O-SA-9	Introduced line	–	–	–

2.3. Agronomic data

Along with BYDV data, data were also recorded at maturity and harvesting of the trial for various agronomic/yield related data. These parameters include number of grains spike^{-1}, number of spikelet's spike^{-1}, spike length, 1000-grain weight grain yield data (kg ha^{-1}), biological yield and harvest index.

2.4. BYDV infection data

Inspection of BYDV appearance was started right from mid of December and after first appearance of BYDV like symptoms, the recording of data was started. Symptoms based scoring of barley yellow dwarf virus was done considering incidence and severity while considering the area covered (Ali and Hodson, 2017), which could be compared to the 0–9 scale). At least two scoring were made for multilocation experiment and several data scoring was made for the trail at Peshawar. The status of BYDV across locations was assessed through comparison of maximum, minimum and overall distribution of BYDV severities at a given location. The resistance status of the tested germplasm was assessed through analyses of their across location score according to the above described scale.

2.5. Aphids infestation

Aphids infestation in the tested germplasm was assessed at Peshawar along with its association with BYDV infection. Aphid infestation data was taken on the tillers according to aphid scouting procedure with the first appearance of aphids on a weekly interval till aphid's disappearance. The BYDV's data was also recorded at the same day as aphid scoring.

2.6. Molecular characterization of diversity

Diversity at the molecular markers (RAPD) level were assessed to know variability in the tested germplasm. According to Ali et al., (2017), genomic DNA was removed from the young leaves using CTAB (acetyl trimethyl ammonium bromide) method. The leaves were crushed in liquid nitrogen and CTAB buffer. All chemicals were prepared according to the protocol (Ali et al., 2017). Gel electrophoresis was used to check the quality of extracted DNA. In addition, Nano drop was used to measure the concentration of DNA extracted for polymerase chain reaction (PCR). For molecular characterization of oat germplasm, a set of around 23 loci were amplified using the selected RAPD primers (Table 2).

2.7. PCR amplification and gel electrophoresis

The thermo scientific PCR kit was used for PCR reactions after the first installed protocol of our group using Biorad Thermo cycler (Naz et al., 2019). For each marker, annealing temperature and other condition were optimized (Table S1). To score the alleles, 2% of agarose gel was used for electrophoresis of the PCR products.

2.8. Data analyses

To create input files for different statistical and population genetics analyses, data were put in MS Excel Sheets. The statistical analysis of the phenotypic data was done through the analysis of variance (ANOVA) techniques using the R-software for morphological and yield parameters. To assess the relationship among various lines based on field data, cluster analyses were conducted in R statistical package. Analyses of molecular data for estimation of diversity and divergence was done in POPPR package of R-software.

3. Results

Our study revealed morphological yield related variability in 16 exotic oat lines and a local check, along with variable level of resistance to BYDV. Molecular markers further identified genetic diversity and level of divergence among these lines.

3.1. BYDV prevalence across locations

The relative prevalence of the disease across the two locations was revealed through the distribution of BYDV infestation on the 17 oat lines as assessed at Peshawar and Kohat. The BYDV incidence and severity varied significantly across locations, whereas the disease was present at both the locations. Although one line had more disease severity at Kohat, the overall distribution of the disease suggested the relatively higher relative prevalence of BYDV at Peshawar compared to Kohat (Fig. 1).

3.2. Temporal variability in BYDV incidence and severity at Peshawar

At Peshawar, BYDV data was taken over the six weekly scoring dates on the 17 oat lines as shown in Fig. 2A. For disease incidence and severity, substantial variability was observed among the genotypes. An overall increase in BYDV incidence was observed for the tested lines. The BYDV incidence was low during the first three weeks (starting from mid of February 2018), when all the varieties shown lower incidence of BYDV, after which an increase in the BYDV incidence occurred. Among the tested lines, SA-O-1 (85%) showed the highest BYDV incidence, for which an increase in BYDV incidence was observed up to the 5th week but at 6th week the disease incidence was reduced. Lowest incidence (48%) was shown by SA-O-10 under the tested conditions at Peshawar. Overviewing the six weeks data, maximum severity was recorded for SA-O-2 (85%), while the minimum was recorded for SA-O-8 (65%) (Fig. 2B).

The overall disease burden was also high as BYDV incidence was higher than 40% on all of the tested genotypes (Fig. 2A). Similarly, the BYDV infestation was important as none of the line could be considered resistant as all of them had severity of more than 50% (Fig. 2B).

Table 2
List of RAPD primer and the amplification conditions used for optimization to molecularly characterize oat germplasm.

Name and Sequence	PCR steps	Temperature checked	Min/sec	Optimized condition
GLA-03 (AGTCAGCCAC)	Denaturation Annealing Extension	95 °C, 95 °C 30–40 °C 72 °C, 72 °C	5 min/30 sec 45 sec 45 sec/7min	32 °C
GLA-04 (AATCGGGCTG)	Denaturation Annealing Extension	95 °C, 95 °C 30–40 °C 72 °C, 72 °C	5 min/30 sec 45 sec 45 sec/7 min	32 °C
GLD-18 (GAGAGCCAAC)	Denaturation Annealing Extension	95 °C, 95 °C 32–40 °C 72 °C, 72 °C	5 min/30 sec 45 sec 45 sec/7 min	32 °C
GLB-05 (TGCGCCCTTC)	Denaturation Annealing Extension	95 °C, 95 °C 32–40 °C 72 °C, 72 °C	5 min/30 sec 45 sec 45 sec/7 min	32 °C

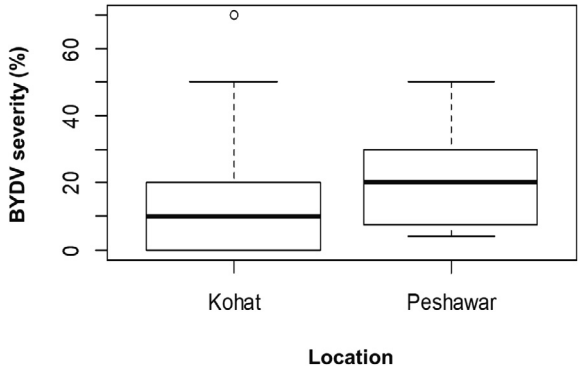

Fig. 1. Incidence of BYDV in exotic oat germplasm tested at Peshawar and Kohat, during the crop season of 2017–18.

Perusal of the weather component revealed an increase in temperature after the first scoring in February 2018. This increase in temperature was observed for the maximum, minimum and average temperatures (Fig. 2C). The variation in humidity was to lesser extent over the crop season, except in November 2017, when the humidity was 62% (Fig. 2C).

3.3. Relative area under disease progress curve

Area under disease progress curve was estimated to determine the individual response of each variety. Based on the scoring dates of BYDV incidence and severity rate, SA-O-2 (100%) covered most of the disease area compared to others varieties. Lines like SA-O-8 (68%), SA-O-13 (69%) and SA-O-16 (70%) had relatively low progression of BYDV infection, though still high to be considered resistant (Fig. 3).

3.4. Aphid infestation and its association with BYDV infection

Association of BYDV with number infected tillers was weak but positive, while with leaf area covered was strongly positive. Oat lines with high aphid infestation showed relatively higher BYDV incidence. Similarly, oat lines with high BYDV incidence had increased number of infected tillers and more leaf area covered with aphids. This reflects on the role of aphids as a vector of BYDV, extensively reported in the literature (Fig. 4A & B).

3.5. Variability for morphological & yield parameters

Analysis of variance showed significant variability among the genotypes with significant genotype over environment interaction. The location interaction was significant for plant height, Leaf area, Panicle length, Panicle weight, Spikelets per panicle, Biological yield, Grain yield and harvest index while the genotype effect was significant for Plant height, leaf area, spikelets per panicle, Biological yield, thousand grain yield, grain yield and harvest index and Genotype by location effect was significant for plant height, leaf area, panicle weight, spikelets per panicle, Biological yield, grain yield and harvest index and Values of CV and LSD are given in Table S2.

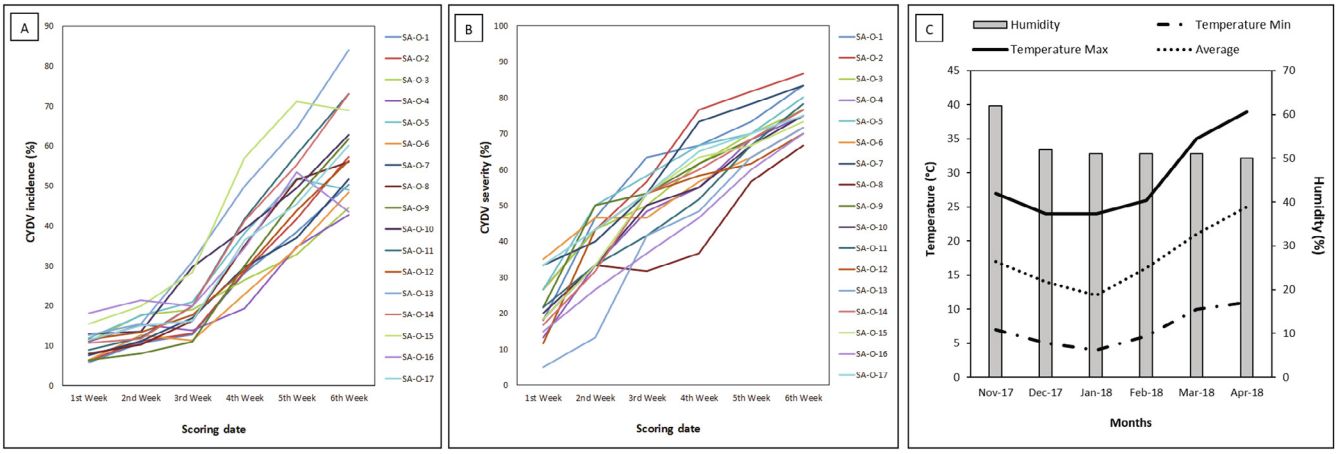

Fig. 2. BYDV incidence (% tillers infected; A) and severity (% leaf area covered; B) progression over six weeks in exotic oat germplasm tested at Peshawar along with the weather parameters (C) during the oat crop season of 2017–18.

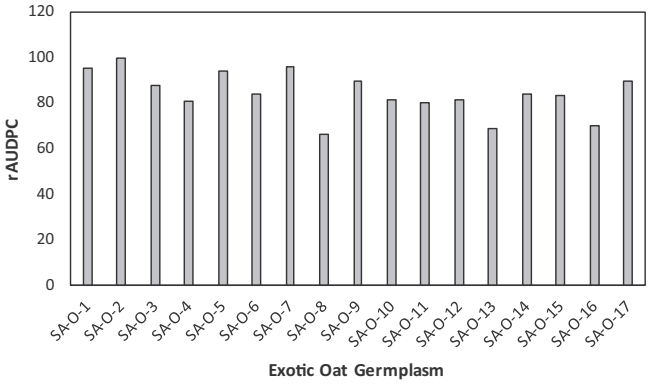

Fig. 3. Area under disease progress curve (AUDPC) based on six scoring dates for exotic oat germplasm tested at Peshawar during 2017–18.

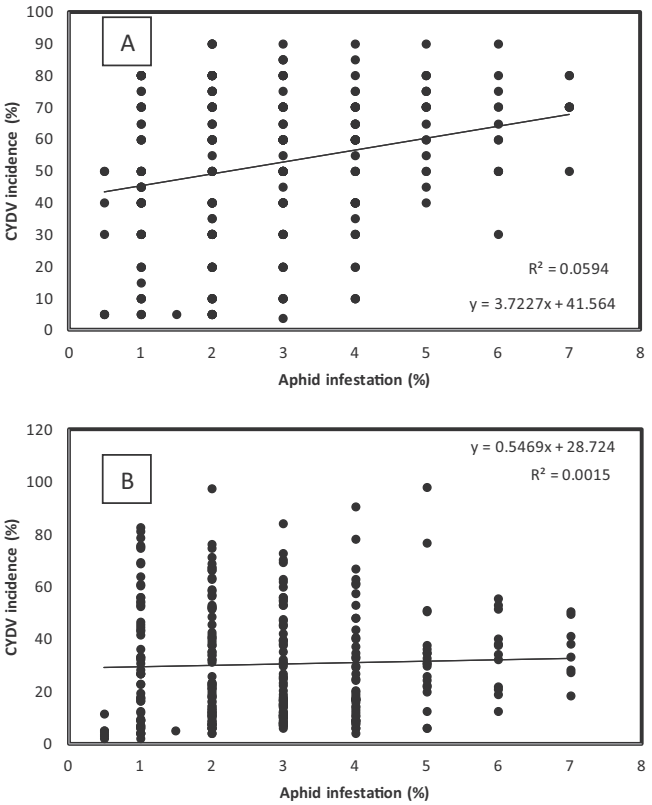

Fig. 4. Association between aphid infestation (%) and BYDV incidence (% tillers infected; A) and severity (% leaf area covered; B) assessed for exotic oat germplasm during 2017–18.

The performances of the tested varieties of Oat at both the locations (Kohat, Peshawar) were highly significant based on ANOVA. The study of the plant height data revealed highly significant performance among the genotypes at both the location (Peshawar, Kohat). At both locations the replication of genotypes also showed highly significant performances (Table 3). The mean plant height value for Kohat was recorded 87 cm, while for Peshawar it was 104 cm recorded. The overall mean value of plant height for both the locations (Kohat, Peshawar) was 95 cm recorded (Table 4).

Comparing the leaf area performances across both locations (Peshawar, Kohat), Peshawar station showed the significantly high performance. Across the location the leaf area performances were highly significant while among the replication, it was variable. At Peshawar station SA-O-11 (69.7 cm^2) gave the maximum leaf area performance, while SA-O-11 (34.0 cm^2) showed the least performance. Overviewing the results at Kohat station, SA-O-15 showed the maximum (52.1 cm^2) leaf area performance, while the minimum performances were recorded for SA-O-8 (30.2 cm^2). The overall mean leaf area value for Kohat was recorded 39.6 cm^2, and for Peshawar 58.6 cm^2 was recorded, while for both the location (Kohat, Peshawar) 49.2 cm^2 recorded. Measuring the panicle length of oat varieties across both the locations showed significant results. The panicle length performances among the genotypes and replication showed variability. Mean panicle length for Kohat and Peshawar was recorded (24 cm) and (22 cm) respectively, while the overall mean panicle length for both the locations was (23 cm) recorded (Table 4).

The performance of the tested varieties in expression of the panicle weight at both locations (Kohat, Peshawar) is shown in the table (Table 4). Perusal of the variance analysis showed highly significant results across the location while among the genotypes and among its replication, it showed variability and significant respectively. The overall mean panicle weight for Kohat was measured (2.38 g), for Peshawar (3.59 g) while for both the location the mean value for panicle weight was measured (2.99 g). Spikelets per panicle for both the locations were counted are shown in the table (Table 4). Perusal of the data analysis showed highly significant results among the genotypes across the location. The mean spikelet's per panicle for Kohat was counted (59), for Peshawar (72) while overall for both the locations (Kohat, Peshawar) was counted (66) (Table 4).

Data on yield parameter of 17 oat lines tested across two location of KP, shows significant progress for yield parameter. Perusal of the variance analysis shows significant results among the tested genotypes while across the location the results were variable (Table 3). Between the tested locations, mean 1000 grain weight for Kohat was measured 39.5 g, for Peshawar it was measured 41.5 g, while for both the location the mean value was measured 39.6 g (Table 5).

Biological yield (g per 0.9 m^2 plot) showed the significant difference between the yield at both the location (Kohat and Peshawar) of Khyber Pakhtunkhwa. The Biological yield among the genotypes across the location was highly significant while the genopytes among replication shows variability (Table 3B). The mean biological yield for Kohat was measured (1098 g) and for Peshawar it was (2068 g). For both the location the mean biological yield was measured (1557 g) (Table 5).

Perusal of the variance data analysis shows significant variation for mean grain yield among the genotypes across the location. Mean grain yield for kohat was recorded (0.155 g) while for Peshawar was (0.231 g) showing the significant difference. The total mean grain yield for both the location was 0.193 g (Table 5).

Highly significant differences for harvest index were observed across the tested locations (Kohat, Peshawar). Mean harvest index recorded for Kohat was17.16%, while for Peshawar it was recorded (11.41%). The overall mean harvest index for both the location was (14.30%). At Kohat station the maximum mean harvest index was recorded for SA-O-10 (49.20%) while the minimum harvest index was observed for Sa-O-2 (11.10%). SA-O-15 at Peshawar station gave the maximum harvest index recorded (16.18%) while minimum was observed for SA-O-3 (8.29%). SA-O-10 gave the maximum mean harvest index (31.18) for both the location while Sa-O-2 was observed giving minimum harvest index (10.37%) for both the locations (Table 5).

The overall grouping of oat lines based on field data as revealed through cluster analyses revealed the presence of at least three groups (Fig. 5). The first group G1, contained only one line SA-O5, the second group "G2" contained six lines including the local line (SA-O17), while the third group "G3" was comprised of 10

Table 3
Mean square values and their significance based on combined ANOVA for morphological and yield parameters in exotic oat lines.

Source of variance	Df	Plant height	Leaf area	Panicle length	Panicle weight	Spikelets per panicle	Biological yield	Thousand grain weight	Grain yield	Harvest index
Location	1	5776**	7458 **	66.44**	24.045**	3466**	29.200**	41.80ns	0.18293**	408.3**
Replication (L)	4	173**	58ns	11.95ns	1.492*	93ns	0.073ns	55.95ns	0.00162ns	15.5ns
Genotypes	16	131**	77**	9.94ns	0.809ns	437**	0.262**	67.20**	0.00897**	67.9**
G × L	16	94**	179**	9.64ns	1.359**	538**	0.315**	29.12ns	0.00830**	53.9**
Residuals	63	30	29	6.86	0.444	64	0.083	20.39	0.00148	17.0

ns = non-significant.
** = significant at <0.001.

Table 4
Data on morphological parameters on oat germplasm tested at two locations of Khyber Pakhtunkhwa (Kohat and Peshawar) during the crop season 2017–18.

Oat genotype	Plant Height (cm)			Leaf Area (cm)			Panicle length (cm)			Panicle weight (g)			Spikelets per panicle		
	Kohat	Peshawar	Mean	Kohat	Peshawar	Mean	Kohat	Peshawar	Mean	Kohat	Peshawar	Mean	Kohat	Peshawar	Mean
SA-O-1	88	101	95	41.0	67.0	54.0	26	22	24	2.53	3.22	2.88	62	86	74
SA-O-10	88	103	95	39.1	56.9	48.0	24	22	23	2.87	2.86	2.87	64	62	63
SA-O-11	88	107	98	34.0	69.7	51.9	26	25	26	3.47	3.31	3.39	67	68	68
SA-O-12	81	101	91	38.3	53.8	46.1	22	20	21	2.13	2.46	2.30	53	56	55
SA-O-13	85	99	92	36.1	55.7	45.9	23	21	22	1.60	3.15	2.38	54	72	63
SA-O-14	89	97	93	37.7	59.4	48.5	24	22	23	2.07	4.02	3.04	61	80	71
SA-O-15	75	96	85	52.1	57.6	54.8	24	21	23	2.10	3.64	2.87	60	70	65
SA-O-16	85	96	91	39.0	57.3	48.2	23	22	22	2.60	2.53	2.57	64	65	65
SA-O-17	81	106	94	44.6	59.1	51.9	25	23	24	1.87	4.53	3.20	52	101	77
SA-O-2	80	115	97	34.2	61.2	47.7	23	24	23	1.73	5.09	3.41	58	94	76
SA-O-3	98	105	102	39.9	62.6	51.2	25	22	24	2.17	3.22	2.69	53	64	59
SA-O-4	94	105	99	38.0	46.0	42.0	25	21	23	2.93	1.97	2.45	62	52	57
SA-O-5	93	102	97	41.1	55.2	48.2	23	23	23	2.73	4.66	3.70	55	90	73
SA-O-6	89	108	98	37.1	61.4	49.2	23	23	23	2.13	3.40	2.77	54	79	67
SA-O-7	88	107	98	44.0	61.5	52.7	23	21	22	2.60	5.33	3.97	70	69	70
SA-O-8	90	107	100	30.2	63.5	50.2	24	21	22	2.80	5.02	4.13	59	74	68
SA-O-9	88	107	97	44.2	48.2	46.2	28	21	24	2.20	2.59	2.39	54	44	49
Mean	87	104	95	39.6	58.6	49.2	24	22	23	2.38	3.59	2.99	59	72	66

Table 5
Data on yield parameters on oat germplasm tested at two locations of Khyber Pakhtunkhwa (Kohat and Peshawar) during the crop season 2017–18.

Oat genotype	1000-grains weight (g)			Biological yield (g per 0.9 m^2 plot)			Grain yield (g per 0.9 m^2 plot)			Harvest index (%)		
	Kohat	Peshawar	Mean	Kohat	Peshawar	Mean	Kohat	Peshawar	Mean	Kohat	Peshawar	Mean
SA-O-1	39.7	37.8	38.8	1188	2517	1853	157	247	202	13.50	10.97	12.23
SA-O-2	34.0	38.2	36.1	866	2183	1525	91	223	157	11.10	9.64	10.37
SA-O-3	41.2	37.7	39.4	997	2417	1707	157	192	175	15.37	8.29	11.83
SA-O-4	37.5	60.7	49.1	883	2150	1517	148	172	160	13.97	6.65	10.31
SA-O-5	37.0	36.3	36.7	3634	2300	2967	145	210	177	14.67	10.94	12.81
SA-O-6	33.6	39.3	36.4	1180	2417	1799	142	277	209	15.99	11.72	13.86
SA-O-7	35.9	42.3	39.1	1050	1517	1284	71	260	216	18.15	10.97	14.56
SA-O-8	36.7	38.3	37.6	1150	2050	1600	198	230	217	19.71	11.73	14.92
SA-O-9	37.8	48.4	43.1	982	1617	1300	210	280	245	17.21	13.05	15.13
SA-O-10	41.7	41.3	41.5	1075	1850	1463	229	263	246	49.20	13.17	31.18
SA-O-11	40.2	42.3	41.2	1227	2183	1705	179	197	188	15.03	10.11	12.57
SA-O-12	34.4	39.1	36.8	0991	2283	1637	118	288	203	15.38	13.57	14.47
SA-O-13	37.0	37.6	37.3	1055	1950	1503	180	177	178	16.19	12.22	14.20
SA-O-14	39.9	37.0	38.5	847	1750	1299	180	197	188	15.66	11.89	13.78
SA-O-15	42.6	45.6	44.1	620	1900	1260	112	270	191	14.11	16.18	15.14
SA-O-16	36.3	42.9	39.6	527	1717	1122	132	192	162	14.61	10.80	12.70
SA-O-17	33.6	41.1	37.4	391	2350	1371	95	252	173	12.77	12.08	12.42
Mean	37.6	41.5	39.6	1098	2068	1557	155	231	193	17.16	11.41	14.26

lines. The better performing line SA-O5 was in G1, while the SA-O10 was grouped in G3.

3.6. Molecular genotyping of oat lines with RAPD markers

Maximum number of multilocus genotypes (MLGs) detection under panmixia were plotted against the number of loci re-sampled confirmed the suitability of RAPD markers to detect the genetic variability in the studied genotypes (Fig. 6A). For the study, between most of the loci, lack of strong linkage also reflected the utility of RAPD markers for the study (Fig. 6B).

Genetic diversity among the 17 exotic oat lines was estimated by four RAPD primers (Table 6). Among the tested molecular markers total of 23 loci were amplified thus giving an average of approximately 1 allele per genotype amplified. All 23 loci were polymorphic and variable level of polymorphism was observed for all loci. Maximum number of Loci was recorded in GLA-04(8), while the minimum number of loci was recorded in GLD-18(4) (Table 6).

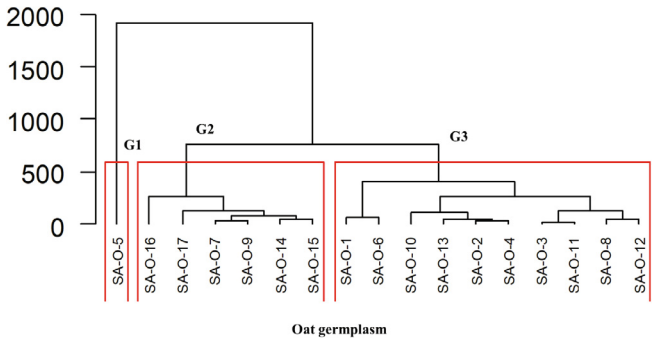

Fig. 5. Clustering of 16 exotic oat lines along with a local check (SA-0-17) based on agronomic traits, yield parameters and BYDV data, tested over two locations of Pakistan.

The maximum gene diversity was recorded (0.529) in loci (GLA-03B230), (GLA-04B130), (GLA-04B300), (GLB-05B150) and (GLA-18B100), while minimum was (0.118) in loci GLA-03B600, GLB-05B330 and GLA-18B500. The maximum Simpsons diversity index was recorded (0.498) for loci GLA-03B230, GLA-04B130, GLA-04B300, GLB-05B150, and GLD-18B100, while minimum was recorded (0.111) in loci GLA-03B600, GLB-05B330 and GLD18B500. The maximum evenness (0.997) was recorded in loci GLA-03B230, GLA-04B130, GLA-04B300, GLB-05B150 and GLD-18B100, while the minimum evenness index (0.497) was recorded in loci GLA-03B600, GLB-05B330 and GLD-18B500 (Table 6).

The neighbor joining tree further also confirmed the overall divergence between the exotic oat and local oat varieties (Fig. 6C). Most of the exotic varieties show divergence from the local variety except for lines SA-O-1, SA-O-15, and SA-O-16, which were in the same clade as the local line (Fig. 6D).

4. Discussion

The current study revealed diversity for morphological and yield parameters in exotic germplasm along with variability for BYDV resistance. The work also revealed substantial genetic diversity as revealed by molecular markers.

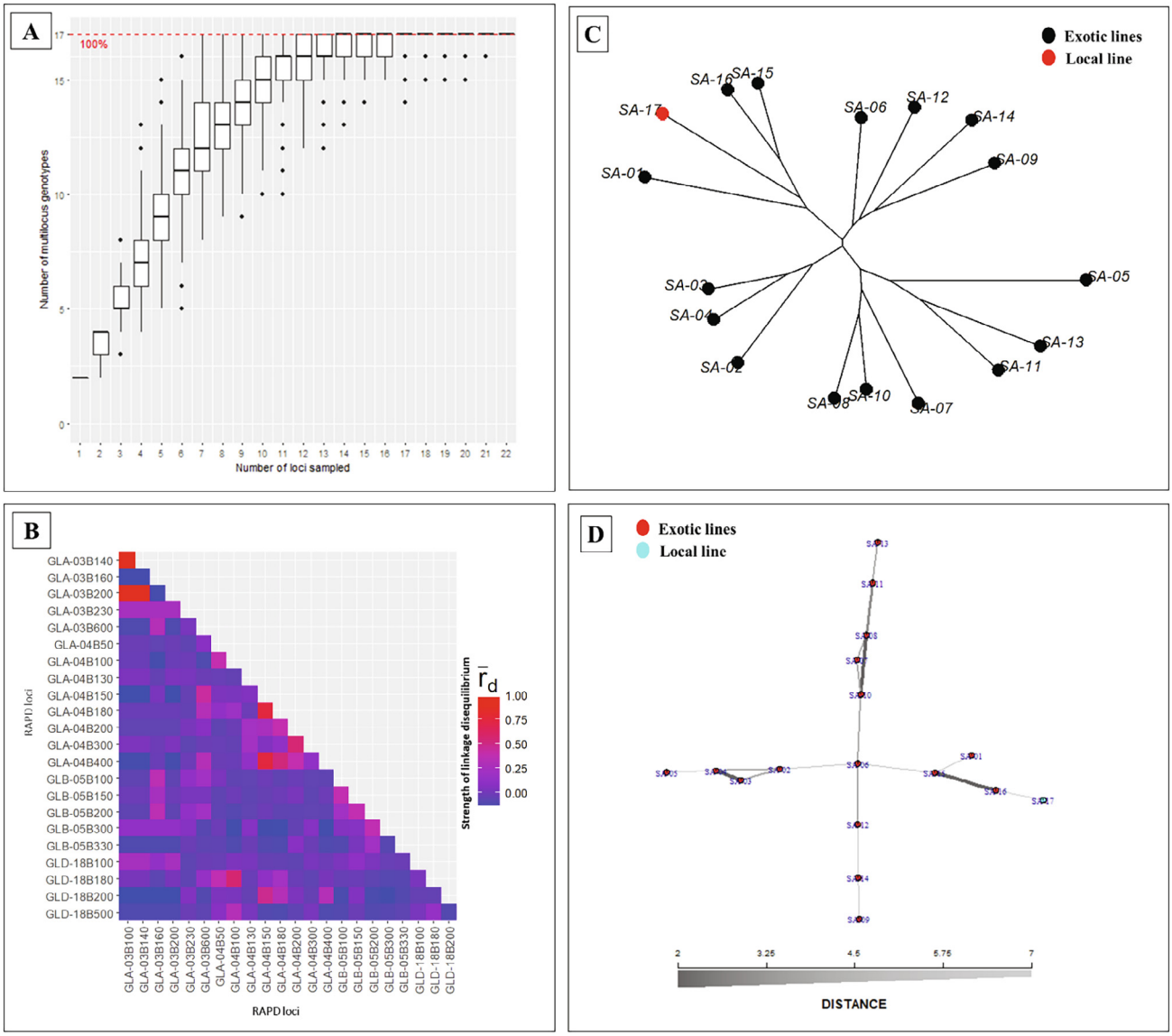

Fig. 6. Feasibility of RAPD loci in terms of multilocus genotype detected against the loci (A) and their association (B); along with the RAPD based genetic relationship in terms of Neighbor-joining (NJ) tree (A) and Network Analyses (B) for oat germplasm.

Table 6
Diversity parameters for RAPD markers amplified in the RAPD germplasm.

RAPD primer	Loci	Gene diversity	Simpsons diversity index	Evenness
GLA-03	**GLA-03B100**	0.382	0.360	0.775
	GLA-03B140	0.382	0.360	0.775
	GLA-03B160	0.382	0.360	0.775
	GLA-03B200	0.382	0.360	0.775
	GLA-03B230	0.529	0.498	0.997
	GLA-03B600	0.118	0.111	0.497
GLA-04	**GLA-04B50**	0.515	0.484	0.970
	GLA-04B100	0.382	0.360	0.775
	GLA-04B130	0.529	0.498	0.997
	GLA-04B150	0.309	0.291	0.690
	GLA-04B180	0.382	0.360	0.775
	GLA-04B200	0.515	0.484	0.970
	GLA-04B300	0.529	0.498	0.997
	GLA-04B400	0.382	0.360	0.775
GLB-05	**GLB-05B100**	0.441	0.415	0.853
	GLB-05B150	0.529	0.498	0.997
	GLB-05B200	0.441	0.415	0.853
	GLB-05B300	0.221	0.208	0.600
	GLB-05B330	0.118	0.111	0.497
GLD-18	**GLD-18B100**	0.529	0.498	0.997
	GLD-18B180	0.485	0.457	0.920
	GLD-18B200	0.309	0.291	0.690
	GLD-18B500	0.118	0.111	0.497

The observed genetic diversity must be useful for oat genetic improvement and broadening the genetic back ground of cultivated oat germplasm. In the past few decades low oat productivity, compared to other fodders has resulted both in the decrease of numbers of grown cultivars and types of oats, along with reduction in acreage. From the genetic resource conservation perspective both of the above factors have negative impact on the farm diversity. Thus assessment of diversity in exotic gene pool is very crucial and important to design and develop new breeding strategies. Various types of molecular and phenotypic markers based studies is increasingly used nowadays to evaluate the genetic diversity of germplasm collections. The usefulness of these markers in such type of analysis has been confirmed for example in wheat, barley and sorghum (Medraoui et al. 2007; Sofalian et al. 2008).

The genetic potential of studied lines for both morphological and yield related parameters could be useful for dual purpose use of this small grain cereal crop, frequently used all over the world for food, feed and fodder. It will increase the cultivation of this crop, particularly in subsistence farming. Global production of the oats decreased 50% in the last fifty years. However, recent demand of oats for human consumption has been gradually increased, particularly owing to its nutritional benefits (Khalil, 2008).

The study revealed the importance of cereal yellow dwarf virus disease at both the locations i.e., Peshawar and Kohat. An overall variable disease pressure was observed with the maximum up to 85% severity and 73% incidence on some lines, though this was non-uniform across locations. BYDV incidence and severity can vary across location, due to variability in environmental conditions (D'Arcy and Burnett, 1995). The disease severity and intensity was high at Peshawar as compared to Kohat, which can be attributed to relatively cold temperature and more rainfall at Peshawar. The disease was also reported to be more effective/progressive in cold temperatures usually at 15–18 °C (D'Arcy and Burnett, 1995). Low temperature and moister are important factors in dispersal of BYDV and its vector aphids (Fabre et al., 2006; Kendall et al., 1992; Thackray et al., 2009).

Variability was observed for grain yield and biological yield across two locations, potentially owing to climatic conditions prevalent at these locations. At Kohat harsh and relatively dry conditions may have inhibited the grain filling of oat especially drought at heading stage (Khalil, 2008). A severe loss of grain in oat has been reported due to the shortage of water (Sandhu and Horton, 1977). At Peshawar, relatively higher yield can be attributed to favorable environmental conditions for the grain filling stage.

The disease did not have significant effect on the 1000-kernel weight (TKW) of the BYDV infection (Baltenberger et al. 1987). This could be due to field-based variability with reliance on natural infection. In a controlled inoculated experiment, comparing the uninfected plants with infected two- or three-leaf stage plant, a slight increase was found in 1000-kernel weight but at later stages of the plant no such effect was found (Goulart et al., 1989). Similarly, in wheat and barley BYDV infected plants showed decrease in the 1000-kernal weight (Baltenberger et al., 1987). The effect of the BYDV infection on the total grain weight in different cereal crops like oats, wheat and barley might be ascribed differently depending on the specific trail conditions and variability in tested germplasm for their level of disease tolerance (Farooq et al., 2019).

Along with morphological and disease resistance-based variability, our results revealed significant variability among the tested lines at the genetic level as assessed through molecular genotyping. Molecular genotyping using various markers are used to evaluate the genetic diversity in cereal germplasm. Different DNA markers are routinely used in the evaluation of genetic diversity (Ali et al., 2014; Naz et al., 2019). Our RAPD markers results revealed high genetic diversity present among the tested lines and had been proved to be an effective tool for germplasm characterization and evaluation of different genotypes. RAPD markers normally used for genotyping due to their simplicity and high level of polymorphism (Belaj et al., 2004; Sesli and Yegenoglu, 2009; Williams et al., 1990). Considering the available resources and academic objectives, we have utilizes RAPD markers based genotyping of Oat germplasm, previously confirmed to be helpful for oat genotypes identification (Hanif et al., 2008).

The RAPD loci identified a high diversity in the studied exotic oat genotypes. Each individual represented a distinct multilocus genotype even when only 15 loci were considered. The exotic oat

genotypes had enough genotypic diversity and gene diversity to be exploited for further genetic improvement (Premkumar et al. 2017). Among the tested RAPD markers a total of 23 alleles were amplified in the tested lines. All the markers showed high level of polymorphism among the 17 proposed oat lines. Polymorphism for RAPD markers has also been reported in oat biotypes collected from different part of Khyber Pahtunkhwa province (Khalil, 2008) and elsewhere (Ruwali et al 2013).

The neighbor joining tree and network analysis further confirmed the overall divergence between the exotic oat and local oat varieties. The local variety i.e. SA-O-17 was closer to SA-O-01, SA-O-15 and SA-O-16, while it was clearly distant from all other exotic lines. Based on the dendrogram analysis, Khalil (2008), also identified two main clusters comprising of Dera Ismail Khan white biotype (representing semi-arid region) and Swat biotype (representing humid region) were most distantly related to each other. Our study further emphasize on the identification of diverse sources of oat germplasm from exotic sources, which may be utilized in on farm diversity management and breeding programs.

5. Conclusions

This study concluded on the genetic diversity and resistance against BYDV in the 16 exotic oat germplasm and 1 local variety. The exotic oat germplasm clearly diverged from the local variety. The local variety (SA-O-17) was found closer to SA-O-1, SA-O-15, and SA-O-16. The overall disease burden was also high as BYDV incidence was higher than 40% on all of the tested genotypes, while none of the line could be considered resistant as all of them had severity of more than 50%. Overall high genetic diversity was observed among the oat varieties, which could be entertained to exploitation in further oat cultivation and improvement. Based on biological yield, SA-O-05 could be recommended for fodder production, while SA-O-10 giving the highest grain yield and harvest index could be recommended for grain production and further breeding programs. The knowledge on molecular diversity must be considered while developing future crosses for genetic improvement of wheat. Future sequencing tools must be used to further elucidate the relationship of exotic oat germplasm with the local variety.

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