Range Mgmt. & Agroforestry 41 (1) : 52-59, 2020
ISSN 0971-2070

Genetic variability studies of advanced generation mutant oat (*Avena sativa* L.) lines for yield, fodder traits and proline content

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Received: 20th March, 2019 Accepted: 24th December, 2019

Abstract
The present study was conducted with 118 mutant oat lines including three checks JO-1, JO-03-91 and Kent which was analyzed based on data of *Rabi* season 2016-17 and *Rabi* 2017-18 to assess the performance, variability, heritability and genetic advance for nineteen yield and its component traits. Analysis of variance revealed significant differences exists among the genotypes for all the characters studied. The estimates of phenotypic coefficient of variation (PCV) were high for dry matter yield per plant per day followed by crude protein yield per plant per day, number of tillers per plant, crude protein yield per plant, number of spikelets per panicle and flag leaf length whereas, values of genotypic coefficient of variation (GCV) were high for crude protein yield per plant per day followed by number of tillers per plant and flag leaf length. High heritability coupled with high genetic advance was recorded for flag leaf width, flag leaf length and thousand seed weight. It was concluded that there is ample scope for exploitation of variation for the traits investigated in these mutant lines for direct selection of high yielding genotypes. Based on genetic variability parameters and proline content for stress resistance some selected putative mutant lines *viz.,* MJO15-6, MJO15-11, MJO15-17, MK15-3, MK15-20, MK15-33, MJ9315-17, MJ9315-32 and MJ9315-46 can be utilized either directly in coordinated trial or as parent in crosses for crop improvement programme to develop high yielding and good quality of fodder oat.

Keywords: Genetic advance, Heritability, Oat, Proline, Variability

Introduction

Oats (*Avena sativa* L.) is an economically important crop used both as fodder for animals and processed oat meal for the human beings. It ranks sixth in world cereal production after wheat, rice, maize, barley and sorghum (FAO, 2012). The global annual oat production over the past five years has ranged from 21 to 24 million metric tonnes (FAOSTAT, 2016). In India, oat is grown on 100,000 hectares of area with the productivity of 35-40 tonnes green fodder per hectare (Anonymous, 2014). It is grown as fodder crop during *Rabi* season in northwestern and central parts of the country and is now even extending to the eastern region as well. Oat (*Avena* spp.) is a cereal crop belonging to the Poaceae family and is considered to have originated in the Mediterranean basin or the Middle East (Murphy and Hoffman, 1992). Cultivated oat (*Avena sativa* L.) is an allohexaploid (2n= 6x= 42) with three diploid progenitor genomes: AA, CC and DD (Baum, 1977). The genome size of oat has been estimated to be 11.3-14.0 GB (Luo *et al.,* 2012).

Lack of adequate variability has been implicated as one of the major limitations in improving the productivity of oat. Genetic variability helps for the choice of the best yielding attributes either for selection or hybridization. As availability and creation of variation in the desired direction is a prerequisite of any crop improvement programme so the induced mutations have been used in plant breeding in many ways. The most direct way is to multiply the seed of the induced mutant and to make it available to growers as soon as enough seed has been produced. The present study was taken to analyze variability, phenotypic and genotypic coefficient of variation, genetic advance and heritability as crop improvement programme over pooled M_4 and M_5 oat mutant lines. Early *in vitro* studies showed that proline can be a reactive oxyzen species (ROS) scavenger. Many of these roles of proline have been discussed in recent reviews (Lehmann *et al*., 2010; Szabados and Savoure, 2010).

Materials and Methods

Experimental site: The present investigation was carried out during *Rabi* season 2016-17 (M_4) and *Rabi* 2017-18 (M_5) under All India Coordinated Research Project on

Forage Crops, at Seed Breeding Farm, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur. Indeed, Jabalpur is situated in 'Kymore plateau and Satpura hills agro-climatic region of Madhya Pradesh, India at 23.91° N latitudes, 79.5° E longitudes and at an altitude of 411.78 m above the mean sea level. The region has sub tropical and semi arid climate. The main features are hot and dry summer and cold winter with occasional showers. The minimum and maximum temperature varies between 6^0 C in January to 45^0 C in May and June months. The average rainfall is about 1200 mm, which is mostly received during July to September. The soil of experimental field was heavy black, clayey, uniform in its topography and free from water logged condition. It was deep, well drained alluvial in origin and had fairly good moisture holding capacity. The crop was grown under normal crop season.

Experimental material: A set of three current and representative oat (*Avena sativa* L.) cultivars namely JO-1 (Jawahar Oat-1), Kent and JO-03-91 (Jawahar Oat-03-91) were selected based on information available on different aspects of forage yield and quality attributes. Seeds of three varieties were procured from AICRP on 'Forage Crops and Utilization' and these seeds were treated with irradiation and chemical mutagens (Table 1) to produce mutant lines. Later a total of 118 mutant lines including three checks were evaluated from pooled M_4 and M_5 generations for genetic variability in different traits.

Observation recorded: The observations were recorded for yield and its component characters *viz*., days to flower initiation, days to 50% flowering, days to maturity, plant height, number of tillers per plant, number of leaves per tiller, panicle length, flag leaf length, internode length, flag leaf width, peduncle length, number of spikelets per panicle, number of florets per panicle, panicle weight, thousand seed weight, dry matter yield per plant per day, crude protein yield per plant, crude protein yield per plant per day and dry matter yield per plant. Based on above genetic parameters some lines were selected and analyzed for proline content according to Bates *et al*. (1973).

Statistical analysis: Analysis of variation including phenotypic and genotypic coefficients of variation was performed according to the method suggested by Burton (1952). Heritability in broad sense was estimated (Hanson *et al*., 1956) and expressed in percentage. Genetic advance as per cent of mean was estimated by the method suggested by Johnson *et al.* (1955).

Table 1. Details of oat varieties and treatments under study

Oat varieties		
JO-1	**Kent**	**JO-03-91**
Gamma irradiation		
Control	Control	Control
200Gy	200Gy	200Gy
250Gy	250Gy	250Gy
300Gy	300Gy	300Gy
350Gy	350Gy	350Gy
400Gy	400Gy	400Gy
450Gy	450Gy	450Gy
500Gy	500Gy	500Gy
Ethyl methane sulphonate (EMS)-3hr		
0.4% EMS	0.4% EMS	0.4% EMS
0.8% EMS	0.8% EMS	0.8% EMS
Gamma radiation + 0.4% EMS- 3hr (combination)		
200Gy + 0.4% EMS	200Gy + 0.4% EMS	200Gy + 0.4% EMS
250Gy + 0.4% EMS	250Gy + 0.4% EMS	250Gy + 0.4% EMS
300Gy + 0.4% EMS	300Gy + 0.4% EMS	300Gy + 0.4% EMS
350Gy + 0.4% EMS	350Gy + 0.4% EMS	350Gy + 0.4% EMS
400Gy + 0.4% EMS	400Gy + 0.4% EMS	400Gy + 0.4% EMS
450Gy + 0.4% EMS	450Gy + 0.4% EMS	450Gy + 0.4% EMS
500Gy + 0.4% EMS	500Gy + 0.4% EMS	500Gy + 0.4% EMS

Table 2. Analysis of variance for different characters of *Avena sativa* L. (pooled)

Sources of variation	Degree of freedom	Mean sum of squares									
		Days to flower initiation	Days to 50 % flowering	Days to maturity	Plant height (cm)	Number of tillers /plant	Number of leaves /tiller	Panicle length (cm)	Flag leaf length (cm)	Internode length (cm)	Flag leaf width (cm)
Replication	2	21.3835**	4.4837	604.5388*	5852.3780**	18.9964**	34.4830**	132.0346**	560.6187**	235.8199**	0.0138
Treatment	117	198.8836**	184.4241**	215.8527**	741.0195**	4.5468**	1.5863**	81.1452**	176.1497**	30.4727**	0.4925**
Error	234	22.308	24.020	61.745	123.740	0.770	0.374	12.277	14.417	6.004	0.057

Sources of variation	Degree of freedom	Mean sum of squares								
		Peduncle length (cm)	Number of spikelets/ panicle	Number of florets per panicle	Panicle weight (g)	Thousand seed weight (g)	Dry matter yield/ plant/ day (g)	Crude protein yield/ plant (g)	Crude protein yield/ plant /day (g)	Dry matter yield/ plant (g)
Replication	2	86.5282**	587.8671	1083.9960	2.0609**	311.1109**	0.9414	0.3198	0.000006	6.1303*
Treatment	117	42.9095**	595.8230**	1098.1877**	0.80020**	60.8447**	0.8975	0.0679	0.000050**	7.8056**
Error	234	18.3729	220.3473	468.0542	0.19927	21.4645	0.8983	0.31591	0.000008	2.0081

*(P<0.05); **(P<0.01)

Results and Discussion

***Analysis of variance*:** Analysis of variance over two years on 118 oat lines showed that genotypes differed significantly indicating a substantial genetic variability in the material for different traits (Table 2). Substantial and exploitable variation in different oat germplasm were also observed earlier by different workers (Chakraborty *et al.,* 2014; Ahmed *et al.,* 2011; Bibi *et al.*, 2012; Arora, 2013; Krishna *et al.*, 2014; Bind *et al.*, 2016). Hence, there is an ample scope for improvement through selection for the traits investigated in the available materials generated through mutation.

***Mean performance and range*:** The mean, range and estimates of phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability in broad sense h^2 (BS) and genetic advance as per cent of mean (GA) of 118 oat lines of pooled over M_4 and M_5 generation for all the characters were recorded (Table 3). Mutant line MJ9315-21 was of late mature type and took 101.17 days for initiation of flowering, while MK15-33 took a minimum of 60.33 days. The overall mean for days to 50 percent flowering in pooled data was recorded to be 88.52 days. Pooled data related to days to maturity ranged from 101.50 days (MJO15-19) to 139.00 days (MJ9315-48). Maximum plant height was recorded for MJ9315-37 (163.00 cm) and minimum for MJO15-3 (102.50 cm). Maximum and minimum number of tillers per plant recorded 3.16 to 10.17, respectively. Pertaining to the number of leaves per tiller the mean value was 5.58 leaves per tiller. Longest panicle length was observed for MJ9315-48 (65.17 cm) and the minimum value was recorded for MJO15-8 (29.17 cm). Longest flag leaf length was recorded in MJO15-32 (56.33 cm), however, shortest flag leaf was in mutant line MJ9315-30 (23.33 cm). Maximum internode length was recorded in MJO15-18 (31.50 cm) and the minimum was in MJ9315-39 (6.67 cm) (Fig 1c). Flag leaf width (Fig 1a and 1b) ranged from minimum width in MJ9315-8 (1.57 cm) to broadest in MJ9315-4 (3.42 cm). Pooled analysis of 2 years revealed that peduncle length had a value between 25.33 cm to 47.50 cm with a mean of 33.17 cm. Maximum peduncle length was observed in mutant line MJ9315-48 (47.50 cm) and MJ9315-21 had minimum peduncle length (25.33 cm).The number of spikelets per panicle varied from 46.67 (MJ9315-8) to 115.33 (MJ9315-14). The maximum number of florets per panicle was present in MJ9315-6 (197.167) whereas it was minimum for MJ9315-8 (93.17). Panicle weight (Fig 1d) varied from 3.65 g (MJ9315-8) to 6.30 g (MJ9315-6) with the mean value of 4.99 g. Range of variation observed for thousand seed weight was 22.45 g to 50.16 g. The overall mean was reported to be 38.89 g. Dry matter yield per plant per day varied from 0.13 g (MJ9315-39) to 0.24 g (MK15-10). Crude protein yield per plant ranges from lowest 1.39 g (MJO15-1) to highest 2.19 g (MJO15-21). The maximum and minimum value of crude protein yield per plant per day was recorded in MJO15-9 (0.02 g) and MJO15-1 (0.01 g), respectively. For dry matter yield per plant, it lied between 17.06 g for MJO15-1 to 25.23 g for MJO15-2.

Among fodder yield and its component characters number of tillers per plant possessed the widest range, followed by number of spikelets per panicle and dry matter yield per plant per day in M_4, M_5 and pooled. This showed that these characters were responsible for wide variation in fodder yield of various genotypes. Wide range of variability was also observed for green fodder yield by Arora *et al.* (2013). Chlorina and yellow viridis type of mutant also observed in M_1 generation (Basha *et al.*, 2015). The results were in agreement with those of Vilaro *et al.* (2004) who reported that thousand-kernel weight ranged from 24.15 to 40.90 g in old varieties, 26.45 to 47.11 g in modern varieties. Similar results were reported by Wildeman (2004) and Buerstmayr *et al.* (2007).

Fig 1. Contrasting variations in leaf (a, b), stem (c) and panicle (d) in mutant lines

Table 3. Variation for genetic parameters in dry matter yield and its components in *Avena sativa* L (pooled data)

Character	Mean	Range		CV		Genotypic variance	Phenotypic variance	Heritability (h^2) %	Genetic advance as % of mean
		Minimum	Maximum	PCV	GCV				
Days to flower initiation	77.15	60.33	101.16	10.67	10.49	45.52	67.83	67.10	14.76
Days to 50 percent flowering	88.51	71.16	109.50	9.14	8.71	41.47	65.49	63.30	11.93
Days to maturity	121.69	101.50	139.00	10.72	3.94	108.04	169.78	63.60	14.04
Plant height (cm)	134.91	102.50	163.00	13.45	10.63	205.76	329.50	62.40	17.31
Number of tillers per plant	5.17	3.16	10.16	28.98	20.71	1.48	2.25	65.80	38.83
Number of leaves per tiller	5.57	4.00	8.83	18.06	9.59	0.64	1.01	63.10	23.48
Panicle length (cm)	38.97	29.16	65.16	16.20	11.65	27.62	39.90	69.20	23.11
Flag leaf length (cm)	35.79	23.33	56.33	23.09	20.51	53.91	68.33	78.90	37.53
Internode length (cm)	23.63	16.66	31.50	16.95	11.36	10.05	16.05	62.60	21.87
Flag leaf width (cm)	2.50	1.56	3.42	17.70	15.40	0.14	0.19	70.90	25.88
Peduncle length (cm)	33.16	25.33	47.50	15.53	8.62	17.18	26.55	64.70	22.15
Number of spikelets per panicle	77.52	46.67	115.33	23.97	14.43	208.12	345.51	60.20	29.75
Number of florets per panicle	134.10	93.16	197.17	19.42	10.81	375.02	678.09	55.30	22.12
Panicle weight (g)	4.98	3.65	6.30	12.67	8.97	0.27	0.40	67.50	17.84
Thousand seed weight (g)	38.89	22.45	50.17	15.12	9.32	28.12	34.59	81.30	25.32
Dry matter yield per plant per day (g)	0.22	0.13	0.31	42.68	7.47	0.002	0.003	66.70	33.53
Crude protein yield per plant (g)	1.740	1.39	2.19	27.75	16.52	0.16	0.23	67.00	38.28
Crude protein yield per plant per day (g)	0.013	0.01	0.02	35.47	28.36	0.0001	0.0001	64.00	46.72
Dry matter yield per plant (g)	21.08	17.06	25.23	9.47	6.59	2.40	3.94	60.90	11.81

CV: Coefficient of variation; PCV: Phenotypic coefficient of variation; GCV: Genotypic coefficient of variation

Phenotypic and genotypic coefficient of variation: The pooled data of two years revealed that phenotypic coefficient of variation ranged from 9.14 per cent (days to 50% flowering) to 42.68 per cent (dry matter yield per plant per day) whereas, genotypic coefficient of variation ranged from 3.94 per cent (days to maturity) to 28.36 per cent (crude protein per plant per day) for different characters under study. The respective phenotypic and genotypic coefficient of variation for various traits were also recorded (Table 1). Similar findings were reported by Bibi *et al.* (2012), Chakraborty *et al.* (2014), Surje and De (2014) for one or more characters. Bind *et al.* (2016) observed low GCV for days to flowering and days to maturity, which confirmed the present findings. Dubey *et al.* (2014) recorded low GCV for leaves per plant and moderate PCV for dry matter yield per plant per day.

Heritability (h^2): Knowledge on the heritability is essential to a plant breeder since it indicates the possibility and extent to which improvement is possible through selection. The broad sense heritability is the relative magnitude of genotypic and phenotypic variances for the traits and it is used for predictive role in selection procedures (Allard, 1960) and it indicates that the character expressing higher heritability estimate are less influenced by environment and such characters also suggest that these are under influence of more number of fixable factors. Burton (1952) suggested that the genotypic coefficient of variation along with heritability estimate would exhibit a better picture of genetic gain expected through phenotypic selection. The highest estimate of broad-sense heritability was recorded for thousand seed weight (81.30%), flag leaf length (78.90%) and flag leaf width (70.90%) in pooled data of M_4 and M_5 generations. Traits with moderate heritability were recorded for panicle length (69.20%), panicle weight (67.50%), days to flower initiation (67.10%), crude protein yield per plant (67.00%), number of tiller per plant (65.80%), dry matter yield per plant per day (66.70%), peduncle length (64.70%), crude protein yield per plant per day (64.00%), days to maturity (63.60%), 50% flowering (63.30%), number of leaves per tiller (63.10%), internode length (62.60%), plant height (62.40%), dry matter yield per plant (60.90%), number of spikelet per panicle (60.20%) and number of florets per panicle (55.30%). High heritability along with high genetic advance for characters like thousand seed weight was observed earlier (Chakraborty *et al.*, 2014; Dubey *et al.*, 2014; Bind *et al.,* 2016). These results indicated the scope for selection in these characters in future improvement programme.

Genetic advance as percentage of mean: The average over the two years highest genetic advance as percentage of mean (at 5% selection intensity) were found for crude protein yield per plant per day (46.72) followed by number of tillers per plant (38.83), crude protein yield per plant (38.28), flag leaf length (37.53), dry matter yield per plant per day (33.53), number of spikelet per panicle (29.751), flag leaf width (25.88), thousand seed weight (25.32), number of leaves per tiller (23.48), panicle length (23.11), peduncle length (22.15), number of florets per panicle (22.12) and internode length (21.87). However, moderate value was recorded for panicle weight (17.84), plant height (17.31), days to flower initiation (14.76), days to maturity (14.03), 50 percent flowering (11.93) and dry matter yield per plant (11.81). Results of the present investigation were also in agreement with earlier studies carried out on oat by Shekhawat *et al*. (2006) and they observed the genetic advance as percentage of mean was maximum for dry fodder yield. Ahmed *et al*. (2013) recorded the traits like leaf width and green fodder yield with high genetic advance. Similar findings for one or more characters in oat were also observed by Bind *et al.* (2016).

Table 4. Proline content (µg per g flw) of selected oat mutant lines

Oat lines	Proline content
JO-1 Check	3.51
MO15-6	18.90
MJO15-11	21.78
MJO15-17	15.44
Kent Check	1.22
MK15-3	5.20
MK15-20	12.00
MK15-33	20.50
JO 03-91 Check	2.46
MJ9315-17	36.71
MJ9315-32	31.61
MJ9315-46	22.77

Proline content: The mutant lines of JO-1 *i.e* MJO15-11, MJO15-6 and MJO15-17 had higher proline contents of 21.78, 18.90 and 15.44 µg per g flw, respectively with respect to check JO-1 (3.51 µg per g flw; Table 4). For the mutant lines of Kent *viz.,* MK15-33, MK15-20 and MK15-3 also had higher proline values of 20.50, 12.00 and 5.20 µg per g flw, respectively against check (1.22 µg per g flw). Similarly mutant lines of JO 03-91 *viz.,* MJ9315-17, MJ9315-32, MJ9315-46 accumulated the higher proline of 36.71, 31.61and 22.77 µg per g flw, respectively against check (2.46 µg per g flw). Overall MJ9315-17 mutant line accumulated the highest proline of 36.71µg per g flw, which was higher than all three checks. These results corroborated with the earlier findings of Mafakeri *et al*. (2010), Caballero *et al.* (2005) and Hu *et al.* (2004).

Conclusion

The analysis of variances of 118 oat lines for all the characters studied showed that mutant lines differed significantly indicating substantial genetic variability in these materials. Higher PCV and GCV were recorded for crude protein yield per plant per day, flag leaf length, number of tillers per plant. High heritability coupled with high genetic advance as a percentage of mean was observed for thousand seed weight, flag leaf length, flag leaf width, crude protein yield per plant per day and panicle length. MJ9315-17, a mutant line accumulated the maximum quantity of proline (36.71µg per g flw) which was higher than all the checks.

Acknowledgement

The authors thank Dr. Sharad Tiwari, Professor and Head, Department of Genetics and Plant Breeding and Director Farm, JNKVV, Jabalpur for providing necessary facilities and constructive suggestions in the research work.

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