doi: 10.17138/TGFT(9)109-119

Research Paper

Salinity tolerance of *Avena sativa* fodder genotypes

Tolerancia de genotipos de avena forrajera a la salinidad

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Abstract

Oats (*Avena sativa* L.) is an important winter season fodder cultivated in many parts of the world. India faces huge shortages of green forage and possesses large salt-affected areas, so identification of salt-tolerant material offers scope for breeding of cultivars for increasing production from salt-affected soils. Forty-eight genotypes of oats comprised of cultivars, germplasm accessions and advanced breeding lines were evaluated with the aim of identifying salt-tolerant genotypes for use on saline soils and/or in programs to breed more salt-tolerant cultivars. Screening was carried out at different growth stages in both pot and field studies. Germination and seedling vigor at different levels of salinity in terms of electrical conductivity (EC), i.e. EC4, EC8, EC12 and EC16, were assessed. Field-level salinity tolerance was assessed in pits where soils had EC ranging from 3.3 to 3.6 dS/m and pH 9.6. Sand culture experiments were carried out on 2 genotypes at different levels of NaCl solution as well as saline soil scrap solution so as to simulate a real field situation. Na, K, Ca and proline concentrations were estimated to understand the mechanism of salinity tolerance of the crop. The study resulted in identification of some suitable genotypes with acceptable levels of salt tolerance, which can be used in developing productive cultivars for saline soils.

Keywords: Diversity, germination, growth, oats, salt stress.

Resumen

En varias partes del mundo la avena (*Avena sativa* L.) es un forraje importante cultivado para la temporada de invierno. India enfrenta una alta escasez de forraje verde y posee grandes áreas afectadas por salinidad, por lo que la identificación de materiales tolerantes a la salinidad ofrece un amplio margen para el mejoramiento de cultivares adaptados con el fin de aumentar la producción en este tipo de suelos. En Jhansi, India, se evaluaron 48 genotipos forrajeros de avena incluyendo cultivares, accesiones de germoplasma y líneas de mejoramiento avanzadas con el objetivo de identificar aquellos tolerantes a la salinidad para uso posterior en suelos salinos y/o en programas de mejoramiento. El estudio se llevó a cabo en diferentes etapas de crecimiento de las plantas, tanto a nivel de invernadero como de campo. Se evaluaron la germinación y el vigor de plántulas a diferentes niveles de salinidad en términos de conductividad eléctrica (CE): CE4, CE8, CE12 y CE16. La tolerancia a la salinidad a nivel de campo se evaluó en fosas llenadas con suelo salino que tenían una CE que variaba entre 3.3 y 3.6 dS/m y un pH de 9.6. Además se realizaron experimentos en arena con dos genotipos a diferentes concentraciones de NaCl, así como en solución de la costra de suelo salino para simular una situación real. También se determinaron las concentraciones de Na, K, Ca y prolina en las plantas para entender el mecanismo de tolerancia a la salinidad. El estudio permitió la identificación de algunos genotipos con niveles aceptables de tolerancia a la salinidad los cuales pueden ser utilizados en el desarrollo de cultivares productivos para suelos salinos.

Palabras clave: *Avena sativa*, crecimiento, diversidad, germinación, estrés por salinidad.

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Introduction

Soil salinity is one of the most important abiotic stresses and is a limiting factor for plant production worldwide (Zhu 2001) with Gao et al. (2016) estimating that more than 6% of the world's total land area is affected by salinity. Saline-sodic soils in India occupy approximately 7% of total land area (1 billion ha) and 20% of the irrigated arable land in arid and semi-arid regions, and this area is increasing (Agarwal et al. 2013). Soil salinity reduces crop growth and its performance, divided into primary and secondary effects. Primary salt effects include metabolic disturbances plus inhibition of growth and development. Secondary salt effects include nutrient deficiency and osmotic dehydration. Soil reclamation and water desalination practices have been implemented in an endeavor to overcome salinity effects but these strategies are very expensive. Developing salt-tolerant lines of plant species appears a feasible option for achieving higher biomass production and yield from salt-affected soils.

Considering the shortage of fodder for livestock in India, it is imperative to develop suitable technologies for increasing fodder production and productivity (Roy et al. 2019a; 2019b) and increasing yields in saline areas would make a valuable contribution. The area under cultivation to produce forage crops has remained constant for the last few decades and there is little prospect of any increase in the area of arable land devoted to forage cultivation. Development of technology to identify suitable genotypes for growing in problem soils, e.g. salt-affected soils, could reduce the forage deficit.

In spite of the fact that forages include much wild and weedy germplasm, which offers better potential for reclamation of saline soils, owing to higher tolerance of biotic and abiotic stresses, there have been limited studies on salt tolerance of forage crops (Maas and Hoffman 1977; Galluzzi et al. 2014; Malaviya et al. 2015; Roy et al. 2019a). Further, efforts to evaluate crops have been restricted to studies of a few selected forages in certain areas. The wide range of genetic variability in many forage genera has not been evaluated in defined conditions.

Oats (*Avena sativa* L.), belonging to the Poaceae family, is grown throughout the world, including Russia, Canada, Poland, Finland, Australia, United States, Spain, United Kingdom, Sweden, Germany and India, for both grain and forage production. The total area cultivated with oats in India is about 0.5 million ha annually. Cultivated oats is an allohexaploid (2n = 6x = 42) derived from 3 ancestral diploid *Avena* genomes (A, C and D). Size of the hexaploid oat genome was found to be 1C = 11.7 pg, which corresponds to 11,443 Mbp (1 pg = 978 Mbp) (Bennett and Leitch 1995). Oat-based food is considered healthy because of the high dietary fiber content of oat groats, particularly beta-glucan (Martínez-Villaluenga and Peñas 2017). It is a popular winter cereal fodder crop grown in north-western and central India and is now planted in the eastern and southern regions. It produces good yields of palatable and nutritious forage.

The genus *Avena* is known for its tolerance of high alkalinity (Holden 1969; Loskutov and Rines 2011) and oats is tolerant of high pH conditions and quite tolerant of salt stress (Zhao et al. 2007; NSW-DPI 2017; Bai et al. 2018). Bhagmal et al. (2009) reported that oats possessed high tolerance of salinity and suggested it could be used for reclamation of saline soils. Salt-tolerance is a complex, multigenic trait and is often a composite response of the integrated biological system. The first step in developing salt-tolerant cultivars of this forage crop would be screening of the wide range of available diverse germplasm lines.

In the present study, oats genotypes, comprised of a few advanced breeding lines, germplasm from Nordic Gene Bank and some existing cultivars, were evaluated under saline conditions and various growth parameters were monitored to identify salt-tolerant lines. Impacts on performance from germination through survival and growth and nutrient concentrations in forage were examined.

Materials and Methods

A series of experiments were conducted in the laboratory and experimental farm of ICAR-Indian Grassland and Fodder Research Institute, Jhansi, India. Forty-eight accessions of oats were used in the study; the list along with their status is presented in Table 1.

In vitro screening for germination and seedling vigor

The 48 genotypes mentioned in Table 1 were evaluated for tolerance of 4 salinity levels, viz. EC4, EC8, EC12 and EC16. One-hundred seeds of each genotype were placed on sterilized filter paper in petri dishes. For Control sets, the filter papers were soaked with distilled water (DW), while for saline treatments, soaking was done with saline water with electrical conductivity (EC) of EC4, EC8, EC12 and EC16 (dS/m or mmho/cm, where 1 dS/m = 1 mmho/cm). Treatment solutions were prepared by dissolving different quantities of NaCl in distilled water so as to get the desired EC level. Data on germination were recorded on Day 7 after soaking by recording the total number of germinated seeds with radicle and plumule growth. Radicle and plumule length were recorded on 3 seedlings in each set on Day15 as a measure of seedling growth.

Table 1. Oats genotypes evaluated for salt tolerance.

SN	Genotype	Details
1	NGB2114	Germplasm
2	NGB2117	Germplasm
3	NGB2118-1	Germplasm
4	NGB2120-1	Germplasm
5	NGB2718	Germplasm
6	NGB4417	Germplasm
7	NGB4467	Germplasm
8	NGB4470	Germplasm
9	NGB4474-1	Germplasm
10	NGB4732	Germplasm
11	NGB4757	Germplasm
12	NGB4758	Germplasm
13	NGB4870	Germplasm
14	NGB4871	Germplasm
15	NGB4872	Germplasm
16	NGB4887	Germplasm
17	NGB6189	Germplasm
18	NGB6368	Germplasm
19	NGB6370	Germplasm
20	NGB6374	Germplasm
21	NGB6963	Germplasm
22	NGB6968	Germplasm
23	NGB6995	Germplasm
24	NGB6997	Germplasm
25	NGB7002	Germplasm
26	NGB7003	Germplasm
27	NGB7007	Germplasm
28	NGB7013	Germplasm
29	NGB7021	Germplasm
30	NGB7026	Germplasm
31	NGB7244	Germplasm
32	NGB7245	Germplasm
33	NGB7247	Germplasm
34	NGB7252	Germplasm
35	NGB7253	Germplasm
36	NGB7259	Germplasm
37	NGB7279	Germplasm
38	JHO2000-3	Advanced breeding line
39	JHO2000-5	Advanced breeding line
40	JHO2001-2	Advanced breeding line
41	JHO2001-4	Advanced breeding line
42	OS6x851-1	Advanced breeding line
43	OS-7x320	Advanced breeding line
44	UPO94xIGO-220	Advanced breeding line
45	JHO822	Cultivar
46	JHO851	Cultivar
47	JHO99-1	Cultivar
48	JHO99-2	Cultivar

Salinity intensity index (SII) was calculated as SII = 1-X_{SS}/X_{NS}, where X_{SS} and X_{NS} are the means for all accessions in salinity-stressed (SS) and non-stressed (NS) environments as per Fisher and Maurer (1978). Salt susceptibility index (SSI) was calculated as SSI = (1-Y_{SS}/Y_{NS})/SII, where Y_{SS} and Y_{NS} are the mean values for a given accession in stressed and non-stressed environments following Bayuelo-Jiménez et al. (2002). Based on SSI values, the genotypes were grouped as susceptible, tolerant and highly tolerant with lower SSI values being an indication of higher tolerance. Standard deviation, Student's t test, 2-factor analysis of variance and regression analyses were performed using MS Excel program.

Sand culture experiment

Seeds of 2 genotypes, JHO2000-5 and OS6x851-1, were sown in pots filled with sand, which had been thoroughly sterilized and washed with distilled water, in 3 replications with 5 treatments including a Control. Salt stress was created by an aqueous solution of NaCl in one treatment. Secondly, in order to simulate the natural salt stress condition, the upper crust of the salt-affected soil from natural condition was scraped, when salts came out on the crust with receding soil moisture, and was then dissolved in distilled water, termed here as saline soil scrap (SSS) solution. Pots were irrigated with one of the following solutions to give the various treatments: (i) distilled water mixed with nutrient solution (Control); (ii) 0.5% NaCl + nutrient solution; (iii) 0.75% NaCl + nutrient solution; (iv) saline soil scrap solution (SSS1) + nutrient solution (EC8 dS/m); and (v) saline soil scrap solution (SSS2) + nutrient solution (EC10.4 dS/m). Twenty-five oat seeds were sown in each pot. The pots were irrigated every day with 500 ml nutrient solution as described by Shannon and Noble (1995) supplemented with salts as described above. After every 5 days the sand in each pot was irrigated with running plain water for 5 minutes to prevent salt build-up. Germination percentage was recorded on Day 10 after sowing and plant survival/ mortality was recorded on Day 25 after sowing. Plant height, leaf length and leaf width were recorded on 3 plants from each pot on Days 30, 45 and 70 after sowing. Three elements (Na, K and Ca) were estimated in plant samples from the various treatments using flame photometry following Jeffery et al. (1989); for this, plants were uprooted, washed in water and blotted dry followed by oven-drying at 80 ºC for 4 days.

Evaluation for field level salinity tolerance

The experiment comprised 44 genotypes, of which 41 genotypes were as per Table 1, except NGB2118-1, NGB4467, NGB4822, NGB4887, NGB7007, NGB7244

and OS-7x320, which were not included due to paucity of seeds. Additionally 3 genotypes, NGB6975, NGB7249 and JHO2001-3, were included. For field-level salinity tolerance, 90–100 seeds of each genotype were sown in rows 50 cm apart in pits in an unreplicated trial due to limited availability of saline pits. The pits were 1 m deep and made of bricks and were filled with natural saline sodic soil collected from nearby villages. Initial EC of the soil was very high, so some salt, which appeared as deposits on the soil surface after irrigation, was removed and the soil homogenized. Final EC of soil in the pits ranged from 3.3 to 3.6 dS/m and the pH was 9.6. Number of germinated seeds and survival of seedlings were recorded 38 days after sowing. Height and leaf dimensions of plants were measured on 3 plants/genotype at 38 days after sowing. Furthermore, proline concentration was estimated for 10 random genotypes and the Control by extraction in 3% sulfosalicylic acid and subsequent colorimetric method of Bates et al. (1973). Proline gets accumulated in plants in response to a variety of abiotic stresses and plays a significant role in stress tolerance, including salt stress (Ashraf and Harris 2004; Ashraf and Foolad 2007; Verbruggen and Hermans 2008).

Results

In vitro screening for germination and seedling vigor

Most genotypes evaluated for germination under saline conditions showed very good germination even at EC16 (Table 2). Mean germination percentages were 92.7, 90.3, 88.8 and 84.2% at EC4, EC8, EC12 and EC16, respectively, compared with 93.9% for Control. Analysis of variance showed significant differences among genotype and treatment means (Table 3). However, the paired t test revealed significant differences only between EC4-EC8 and EC12-EC16 but no differences between DW-EC4 and EC8-EC12 (Table 2). Thus, high EC created by NaCl had some effect on germination. A few genotypes, which showed low germination percentage under saline conditions, also had poor germination in Control treatments (Table 2). Some genotypes showed numerically higher germination than the Control, and their susceptibility index showed a negative value. The regression equation for germination percentage (y) against electrical conductivity of the germination fluid (x) was: y = 94.62 - 0.58**x.

Mean radicle growth of these genotypes was reduced to 5.5 and 4.8 cm, respectively, at EC12 and EC16 compared with 6.6 cm in Control, although radicle growth at EC4 and EC8 was higher than that in Control. The t test showed significant differences between DW-EC4, EC4-EC8, EC8-EC12 and EC12-EC16. The regression equation for radicle length (y) against EC of irrigation water (x) was: y = 7.67 - 0.16**x. Twenty-four genotypes possessed negative SSI values, indicating their salinity-tolerant nature, although most genotypes showed reduced growth at EC12 and EC16.

Mean plumule growth was a little higher at EC4 but reduced to 3.5, 2.6 and 2.2 cm at EC8, EC12 and EC16, respectively, compared with 3.8 cm in distilled water. Twenty-one genotypes displayed salinity-tolerant nature for plumule growth as indicated by negative SSI values. A t test revealed no difference between growth in DW-EC4 and that in EC12-EC16. The regression equation for plumule growth (y) against EC of irrigation water (x) was: y = 4.24 - 0.12**x. Analysis of variance established significant differences among genotypes as well as treatments (Table 3). Twenty genotypes possessed salinity-tolerant nature for both radicle and plumule growth.

Sand culture experiment

Germination percentages at 10 days after sowing fell in the following ranges: 88–92% for Control; 88–92% for 0.5% NaCl; 72–84% for 0.75% NaCl; 92% in scrap soil solution (SSS1) with EC8.0 dS/m; and 60–92% in scrap soil solution (SSS2) with EC10.4 dS/m for JHO2000-5. For OS6x851-1, germination percentage was: 92–96% for Control; 100% for 0.5% NaCl treatment; 88–100% for 0.75% NaCl; 96–100% for SSS1; and 84–100% for SSS2. At Day 25 after sowing, most plants in 0.5 and 0.75% NaCl treatments had survived, while one-third had died in EC8.0 dS/m and two-thirds in EC10.4 dS/m. By Day 45 after sowing, no seedlings in SSS1 and SSS2 survived. Plant height was drastically reduced at EC8.0 and EC10.4 by Day 25 after sowing, whereas there was little effect on height in 0.5 and 0.75% NaCl treatments relative to Control. A similar trend was observed for leaf length and leaf width (Table 4). Even at Day 70 after sowing, seedlings in 0.5 and 0.75% NaCl treatments were growing well, but JHO2000-5 seedlings were 15–28% shorter than those in Control, and OS6x851-1 seedlings were 26–35% shorter than Control plants. Analysis of variance revealed significant differences among treatments and the morphological attributes of the plants. The interaction effects were also significant (Table 3).

Table 2. Germination, radicle length and plumule length in *Avena sativa* genotypes growing at different levels of salinity.

Genotype	Germination (%)						Radicle length (cm)						Plumule length (cm)					
	DW[1]	EC4	EC8	EC12	EC16	Av SSI	DW	EC4	EC8	EC12	EC16	Av SSI	DW	EC4	EC8	EC12	EC16	Av SSI
NGB2114	95.0	100.0	100.0	90.0	90.0	-0.1	2.7	6.9	4.9	7.2	3.0	-6.9	1.6	3.2	2.8	3.0	1.0	-6.2
NGB2117	100.0	100.0	100.0	100.0	100.0	0.0	6.4	7.0	7.7	5.2	9.0	-0.8	3.8	3.6	1.6	0.7	4.8	0.9
NGB2118-1	85.0	70.0	80.0	100.0	70.0	0.4	3.9	9.1	7.1	3.9	3.4	-3.9	1.4	4.5	2.9	1.1	1.5	-6.4
NGB2120-1	90.0	100.0	100.0	100.0	95.0	-1.6	8.3	7.7	6.4	4.1	6.8	1.7	3.5	3.1	5.7	1.3	3.1	0.2
NGB2718	100.0	100.0	100.0	90.0	95.0	0.6	9.2	10.1	7.6	4.3	4.4	1.7	7.2	6.2	3.4	0.7	0.4	2.6
NGB4417	100.0	100.0	100.0	95.0	95.0	0.4	4.6	6.3	4.5	4.0	4.2	-0.1	3.8	3.4	2.7	2.5	0.2	1.0
NGB4467	90.0	95.0	95.0	85.0	75.0	0.2	5.8	8.7	6.7	3.6	2.9	0.0	5.6	2.8	2.9	2.1	1.8	1.9
NGB4470	85.0	85.0	75.0	80.0	80.0	1.2	11.9	7.3	7.7	6.1	3.5	2.9	6.2	3.3	3.3	3.5	2.0	3.2
NGB4474-1	100.0	100.0	100.0	90.0	85.0	0.8	7.0	8.5	4.5	5.0	4.5	1.6	5.5	5.8	1.4	2.3	1.8	2.4
NGB4732	90.0	85.0	80.0	100.0	100.0	0.1	7.9	8.6	5.6	3.4	5.3	2.0	5.3	2.7	1.4	1.0	2.0	3.4
NGB4757	95.0	95.0	80.0	80.0	70.0	2.4	3.7	8.7	8.0	2.8	3.7	-4.7	1.8	4.9	3.9	3.1	2.8	-6.9
NGB4758	100.0	100.0	95.0	85.0	90.0	1.3	6.3	8.0	6.2	4.6	5.1	0.3	1.5	3.1	2.3	0.1	0.1	-1.6
NGB4870	100.0	95.0	100.0	80.0	80.0	1.5	7.6	8.4	5.2	6.9	2.3	1.6	4.3	3.5	2.2	3.1	2.1	2.2
NGB4871	100.0	100.0	100.0	100.0	100.0	0.0	5.5	7.2	8.5	5.6	5.3	-2.1	3.0	1.3	3.4	0.7	0.8	0.0
NGB4872	85.0	75.0	70.0	45.0	60.0	4.3	3.6	7.3	7.1	5.4	4.1	-5.1	1.9	2.3	3.6	2.1	0.7	-4.2
NGB4887	100.0	100.0	100.0	80.0	70.0	1.7	5.3	10.6	9.2	4.3	1.7	-2.4	2.5	7.9	5.4	4.6	1.9	-6.3
NGB6189	15.0	10.0	20.0	20.0	20.0	-3.9	6.3	4.1	4.3	5.9	3.4	1.9	1.5	1.3	1.1	0.9	0.7	1.9
NGB6368	100.0	100.0	90.0	100.0	95.0	0.8	6.8	9.3	7.6	9.8	5.4	-1.3	6.7	6.7	7.6	9.8	2.9	-0.8
NGB6370	100.0	95.0	100.0	95.0	100.0	0.3	4.7	6.9	7.2	4.2	10.1	-3.0	2.1	4.1	3.6	1.8	8.7	-4.9
NGB6374	85.0	70.0	60.0	85.0	75.0	2.6	9.9	7.1	5.9	5.4	3.8	2.8	4.6	3.5	2.2	3.5	0.9	2.9
NGB6963	100.0	100.0	85.0	70.0	80.0	2.9	4.8	8.8	9.7	6.8	9.7	-5.6	2.3	6.5	6.6	6.3	6.2	-9.7
NGB6968	100.0	70.0	90.0	70.0	80.0	3.1	6.5	4.6	7.1	7.2	3.1	0.4	3.1	1.0	3.8	2.4	2.1	0.8
NGB6995	100.0	100.0	95.0	80.0	75.0	1.9	8.8	9.6	6.0	3.7	5.1	2.2	5.0	4.8	1.0	3.6	2.5	2.8
NGB6997	100.0	100.0	100.0	100.0	95.0	0.1	7.6	7.9	7.8	4.7	3.7	0.9	4.1	2.7	5.5	1.9	1.1	0.7
NGB7002	95.0	100.0	100.0	95.0	90.0	-0.3	6.9	11.3	7.7	6.8	5.7	-0.8	3.9	8.3	6.4	2.4	1.9	-2.7
NGB7003	95.0	100.0	100.0	95.0	100.0	-0.6	9.1	8.9	9.9	5.8	5.1	0.7	5.6	5.2	7.2	2.0	3.1	0.3
NGB7007	100.0	100.0	95.0	85.0	55.0	2.1	7.9	7.2	6.2	3.0	2.6	2.3	3.8	3.2	2.8	1.8	1.7	2.2
NGB7013	95.0	95.0	100.0	100.0	95.0	-0.6	7.1	6.9	4.5	5.2	5.0	1.9	4.4	3.9	2.4	2.9	2.3	2.3
NGB7021	100.0	100.0	100.0	95.0	95.0	0.4	4.1	6.5	7.0	5.2	2.7	-2.9	3.4	2.9	2.8	1.0	0.6	-0.4
NGB7026	100.0	95.0	75.0	95.0	85.0	2.4	5.1	9.0	5.4	4.0	3.5	-0.3	4.1	4.7	1.0	0.9	0.5	1.5
NGB7244	45.0	55.0	40.0	50.0	40.0	0.0	3.8	7.4	7.6	4.1	3.0	-3.9	3.8	2.9	4.1	3.2	2.1	-1.6
NGB7245	100.0	90.0	90.0	95.0	90.0	1.3	6.9	6.5	6.8	5.8	4.4	0.7	2.6	2.6	1.6	0.5	0.3	1.6
NGB7247	80.0	95.0	90.0	85.0	90.0	-1.8	3.8	7.2	5.2	6.6	6.7	-3.9	4.3	4.6	2.8	1.1	2.5	-1.1
NGB7252	100.0	95.0	90.0	85.0	90.0	1.7	4.6	8.6	8.2	7.9	6.7	-4.9	2.3	3.3	3.6	5.5	3.1	-4.5
NGB7253	100.0	100.0	100.0	100.0	100.0	0.0	5.0	6.9	8.8	6.8	6.9	-3.7	2.8	3.8	2.4	2.4	2.0	-1.9
NGB7259	100.0	100.0	100.0	95.0	80.0	0.7	6.7	8.6	7.4	5.7	3.1	0.1	4.4	4.1	4.6	3.2	2.9	0.2
NGB7279	85.0	90.0	65.0	80.0	50.0	2.7	7.9	7.2	6.8	4.5	4.6	1.6	3.3	3.0	4.6	1.4	1.1	0.7
JHO2000-3	100.0	100.0	100.0	100.0	95.0	0.1	6.8	8.1	7.0	6.4	4.6	0.1	2.9	3.8	2.2	2.3	0.7	0.4
JHO2000-5	100.0	100.0	95.0	100.0	100.0	0.3	6.9	9.7	7.3	6.8	7.7	-0.7	3.5	7.4	5.0	4.2	5.1	-2.7
JHO2001-2	100.0	95.0	95.0	95.0	90.0	0.9	5.3	11.4	8.4	9.2	1.7	-3.6	4.2	5.0	3.3	3.7	3.1	-1.6
JHO2001-4	100.0	100.0	100.0	100.0	80.0	0.5	8.1	8.6	10.2	7.7	2.5	-0.2	3.2	7.3	6.7	4.7	1.8	-3.6
OS6x851-1	100.0	100.0	100.0	100.0	90.0	0.2	7.4	8.5	9.2	5.9	5.5	-0.4	4.0	4.5	5.4	2.3	1.0	-0.6
OS-7x320	100.0	100.0	100.0	95.0	95.0	0.4	9.3	10.2	8.1	8.2	7.6	0.7	6.9	5.7	3.2	4.2	3.4	1.8
UPO94xIGO-220	100.0	95.0	85.0	95.0	60.0	2.3	9.2	7.5	5.0	4.1	2.6	3.1	3.8	3.1	2.3	2.1	1.8	2.8
JHO822	100.0	100.0	100.0	100.0	100.0	0.0	7.3	8.9	9.6	4.0	9.4	-0.8	3.4	5.0	4.1	2.0	5.0	-1.3
JHO851	100.0	100.0	100.0	100.0	95.0	0.1	4.0	11.7	8.0	6.6	4.0	-6.2	6.3	6.5	4.0	2.7	1.7	-2.1
JHO99-1	95.0	100.0	100.0	100.0	100.0	-0.8	8.5	7.7	8.1	5.9	6.9	0.9	4.5	5.2	4.4	2.6	4.2	0.5
JHO99-2	100.0	100.0	100.0	100.0	100.0	0.0	8.8	7.9	5.6	4.2	2.9	2.7	3.8	3.1	3.1	2.7	2.2	2.1
Mean	93.9	92.7	90.3	88.8	84.2		6.6	8.1	7.1	5.5	4.8		3.8	4.2	3.5	2.6	2.2	
Min	15.0	10.0	20.0	20.0	20.0		2.7	4.1	4.3	2.8	1.7		1.4	1.0	1.0	0.1	0.1	
Max	100.0	100.0	100.0	100.0	100.0		11.9	11.7	10.2	9.8	10.1		7.2	8.3	7.6	9.8	8.7	
SD	14.9	15.8	16.5	16.0	17.4		2.0	1.5	1.5	1.6	2.1		1.5	1.7	1.7	1.7	1.7	
t test probability		0.13	0.02	0.16	0.00			0.00	0.00	0.00	0.02			0.09	0.01	0.00	0.10	
SII		0.01	0.04	0.05	0.10			-0.24	-0.08	0.16	0.27			-0.10	0.07	0.33	0.42	
Regression			Y=94.62-0.58**x						Y=7.67-0.16**x						Y=4.24-0.12**x			

[1]DW = distilled water (Control); EC4 = electrical conductivity 4 dS/m; EC8 = electrical conductivity 8 dS/m; EC12 = electrical conductivity 12 dS/m; EC16 = electrical conductivity 16 dS/m; SSI = salt susceptibility index; SII = salinity intensity index.

Table 3. ANOVA table for various traits of oats genotypes grown in distilled water and solutions with EC4, EC8, EC12 and EC16.

Source of Variation	SS	df	MS	F	F crit
Seed germination (in vitro)					
Genotype	51,299.58	47	1,091.48	20.89**	1.43
Treatment	2,777.71	4	694.43	13.29**	2.41
Error	9,822.29	188	52.25		
Total	63,899.58	239			
Radicle length (in vitro)					
Genotype	209.92	47	4.46	1.61**	1.43
Treatment	337.90	4	84.48	30.41**	2.42
Error	522.31	188	2.78		
Total	1,070.14	239			
Plumule length (in vitro)					
Genotype	252.72	47	5.38	3.02**	1.45
Treatment	66.60	3	22.208	12.46**	2.67
Error	251.22	141	1.788		
Total	570.55	191			
Na, K and Ca concentration (sand culture)					
Genotype	546.20	5	109.24	18.06**	2.48
Treatment	9,121.55	2	4,560.78	754.19**	3.26
Interaction	3,549.09	10	354.91	58.6**	2.11
Error	217.70	36	6.047		
Total	13,434.54	53			
Morphological attributes (sand culture)					
Genotype	923.84	9	102.60	3.45**	1.97
Treatment	139,027.00	4	34,757.00	1,168.00**	2.46
Interaction	27,747.57	36	770.80	25.90**	1.53
Error	2,974.62	100	29.75		
Total	170,673.04	149			

Table 4. Germination, mortality and morphological attributes of 2 oats genotypes growing in sand culture irrigated with solutions containing varying salt levels.

Genotype	Treatment	MG[2]	Mortality[3]	30 days after sowing			45 days after sowing			70 days after sowing		
		(%)	(%)	Height (cm)	LL[4] (cm)	LW[5] (cm)	Height (cm)	LL (cm)	LW (cm)	Height (cm)	LL (cm)	LW (cm)
JHO2000-5	0.50% NaCl	90.7	1.5	30.0	23.7	0.8	42.0	29.3	1.3	51.9	28.2	1.3
	0.75% NaCl	80.0	0.0	24.3	18.1	0.5	34.1	26.1	1.1	45.4	23.4	1.1
	SSS1[1]	92.0	39.1	16.8	13.2	0.3	0.0	0.0	0.0	0.0	0.0	0.0
	SSS2	72.0	70.1	13.0	10.3	0.2	0.0	0.0	0.0	0.0	0.0	0.0
	Control	90.7	0.0	37.7	29.1	1.0	51.8	34.0	1.5	61.3	32.7	1.4
OS6x851-1	0.50% NaCl	100.0	2.7	31.3	23.7	0.8	36.6	21.4	1.2	52.5	26.5	1.4
	0.75% NaCl	93.3	0.0	26.9	20.8	0.7	40.8	25.8	1.2	47.4	22.6	1.4
	SSS1	97.3	28.7	11.1	9.1	0.4	0.0	0.0	0.0	0.0	0.0	0.0
	SSS2	88.0	64.5	6.5	5.3	0.3	0.0	0.0	0.0	0.0	0.0	0.0
	Control	93.3	0.0	35.5	27.5	1.2	50.8	36.0	1.4	72.9	36.2	1.8

[1]SSS1 = Saline soil scrap solution, EC8.0 dS/m; SSS2 = Saline soil scrap solution, EC10.4 dS/m; [2]MG = Mean germination; [3]25 days after sowing; [4]LL = Leaf length; [5]LW = Leaf width.

Plants irrigated with 0.5 and 0.75% NaCl solution showed concentrations of Na ranging from 36.5 to 43 mg/g DM compared with 7.2–14.9 mg/g DM in Control (Table 5), while concentrations of K ranged from 17.4 to 25.5 mg/g DM for saline treatments and 26.2–34.7 mg/g DM for Control. Concentrations of Ca ranged from 0.26 to 0.33 mg/g DM in saline conditions and 0.12–0.14 mg/g DM in Control (Table 5). Analysis of variance revealed significant differences among treatments and for the accumulation of Na, K and Ca in plants growing in saline

and Control treatments. The interaction effects were also significant (Table 3).

Proline concentrations in leaves increased with increasing salt concentration and mean values increased from 0.036 × 10^{-5} µg/g fresh weight for Control to 0.166 × 10^{-5} µg/g fresh weight for 0.75% NaCl treatment (Table 5).

Evaluation for field level salinity tolerance

Of the 44 genotypes sown into saline soil (i.e. at EC3.3 and pH 9.6) as well as normal non-saline soil condition (Control), NGB7259 showed highest germination percentage (>75%), whereas 5 genotypes, viz. NGB2718, NGB2120-1, NGB7002, NGB7253 and JHO2001-4, showed 50–75% germination. Twenty-seven genotypes showed 25–50% germination, while 11 genotypes showed less than 25% germination (Table 6). Growth of the plants was drastically impacted and only NGB7259 recorded >50% survival, with the remaining genotypes showing <20% survival. Morphological observations at Day 38 after sowing revealed that most genotypes had poor growth at this salinity level. Eleven genotypes (NGB6368, NGB6968, NGB6995, NGB7003, NGB7013, NGB7021, NGB7245, NGB7247, NGB7252, JHO2001-2, JHO851) showed 100% mortality. Hence, survival percentage and growth parameters presented in Table 7 are for 33 genotypes only. The mean survival of plants in the saline treatment was only 16.3% compared with 76.6% in Control. Plant growth was adversely affected and the average plant height of the genotypes growing under salt stress was 7.2 cm compared with 52.5 cm in non-stressed Control condition (Table 7). Leaf growth was also badly affected and mean leaf length and width in saline soil were 6.3 and 0.4 cm, respectively, compared with 38.0 and 1.3 cm in Control (Table 7). Proline accumulation was higher among plants growing under saline conditions than in Control (Table 8).

Table 5. Na, K, Ca and proline concentrations in 2 oats genotypes growing in Control and saline conditions in sand culture.

Genotype	Treatment (% NaCl)	Na (mg/g dry wt)	K (mg/g dry wt)	Ca (mg/g dry wt)	Proline (µg/g fresh wt)
JHO2000-5	0.50	43.04	17.40	0.31	0.064 ×10^{-5}
	0.75	41.83	19.75	0.26	0.115 ×10^{-5}
	Control	14.87	26.18	0.14	0.034 ×10^{-5}
OS6x851-1	0.50	36.50	21.92	0.33	0.058 ×10^{-5}
	0.75	38.75	25.54	0.29	0.217 ×10^{-5}
	Control	7.17	34.67	0.12	0.039 ×10^{-5}

Table 6. Grouping of oats genotypes based on germination at EC3.3 and pH 9.6 (field tolerance).

Germination percentage			
<25%	25–50%	50–75%	>75%
NGB4470, NGB4871, NGB4872, NGB6189, NGB6370, NGB6975, NGB7013, NGB7249, NGB7252, NGB7279, JHO2000-3	NGB2114, NGB2117, NGB4417, NGB4467, NGB4474-1, NGB4732, NGB4757, NGB4758, NGB4870, NGB6368, NGB6374, NGB6963, NGB6968, NGB6997, NGB7003, NGB7021, NGB7026, NGB7247, JHO2001-2, JHO2000-5, OS6x851-1, OS-7x320, UPO94xIGO-220, JHO822, JHO851, JHO99-1, JHO99-2	NGB2120-1, NGB2718, NGB7002, NGB7253, JHO2001-4	NGB7259

Table 7. Survival percentage and morphological attributes of oats genotypes grown in soil at EC3.3 and pH 9.6 (stressed, field tolerance) as well as in Control soil, at 38 days after sowing.

Genotype	Surviving plants (%)		Plant height (cm)		Leaf length (cm)		Leaf width (cm)	
	Stressed	Control	Stressed	Control	Stressed	Control	Stressed	Control
NGB2114	24.5	72.0	7.3	60.8	6.2	44.9	0.39	1.27
NGB2117	3.5	68.0	4.8	52.7	4.4	40.4	0.30	1.13
NGB2120-1	7.0	92.0	5.8	36.8	5.3	24.8	0.37	1.20
NGB2718	31.0	68.0	7.6	59.1	6.7	34.3	0.43	1.20
NGB4417	4.0	92.0	4.1	45.6	4.0	32.9	0.33	1.47
NGB4470	9.7	32.0	6.3	36.5	5.4	34.6	0.31	1.32
NGB4474-1	2.0	88.0	5.0	47.7	4.7	33.1	0.35	1.43
NGB4732	24.0	80.0	7.4	58.6	6.5	38.4	0.34	1.12
NGB4757	12.0	60.0	4.3	31.3	3.6	32.6	0.27	1.10
NGB4758	10.0	80.0	7.6	64.2	7.0	44.9	0.37	1.23
NGB4870	12.0	72.0	6.9	42.1	5.9	30.2	0.35	0.90
NGB4871	14.0	96.0	6.2	55.5	5.9	36.2	1.53	1.24
NGB6189	2.0	8.0	6.8	55.5	5.8	40.7	0.30	1.30
NGB6370	7.0	52.0	7.3	54.6	6.2	39.9	0.30	1.63
NGB6374	10.0	40.0	4.6	35.7	4.2	27.1	0.29	0.80
NGB6963	35.5	84.0	9.6	47.9	8.8	36.7	0.48	0.97
NGB6975	7.0	80.0	9.6	65.0	7.6	44.2	0.40	1.70
NGB6997	17.0	88.0	7.3	64.6	6.2	48.1	0.28	1.18
NGB7002	22.0	76.0	6.8	44.8	6.3	32.4	0.40	0.93
NGB7026	22.7	84.0	8.1	61.0	7.3	43.9	0.45	1.50
NGB7249	10.0	92.0	7.7	46.0	7.4	42.0	0.33	1.07
NGB7253	9.0	96.0	4.2	57.4	3.6	39.7	0.30	1.37
NGB7259	57.0	100.0	13.5	58.8	10.7	46.3	0.57	1.07
NGB7279	6.5	36.0	4.7	49.6	4.0	36.3	0.27	1.38
JHO2000-3	4.0	72.0	6.4	42.6	5.4	33.6	0.28	1.07
JHO2000-5	10.0	84.0	6.2	54.9	5.7	39.2	0.30	1.30
JHO2001-3	11.0	76.0	12.8	55.4	10.7	35.6	0.48	0.98
JHO2001-4	21.0	100.0	8.0	56.4	6.8	39.9	0.51	1.60
OS6x851-1	9.0	100.0	5.8	54.1	5.4	36.8	0.57	1.67
UPO94xIGO-220	39.0	68.0	8.8	57.5	7.9	40.9	0.47	1.30
JHO822	42.0	96.0	10.7	61.9	8.7	43.3	0.47	1.20
JHO99-1	15.0	96.0	6.7	60.7	5.4	42.4	0.50	1.40
JHO99-2	27.0	100.0	8.1	55.9	7.0	38.0	0.47	1.47
Mean	16.3	76.6	7.2	52.5	6.3	38.0	0.42	1.26
Min	2.0	8.0	4.1	31.3	3.6	24.8	0.27	0.80
Max	57.0	100.0	13.5	65.0	10.7	48.1	1.53	1.70
SD	12.95	22.20	2.23	9.03	1.76	5.48	0.22	0.23

Table 8. Estimation of proline concentration among oats genotypes growing in Control and saline soil.

Genotype	Proline (µmol/g fresh weight)	
	Control soil	Saline soil
NGB2718	0.074×10^{-5}	0.079×10^{-5}
NGB4470	0.038×10^{-5}	0.084×10^{-5}
NGB4732	0.045×10^{-5}	0.057×10^{-5}
NGB6370	0.071×10^{-5}	0.313×10^{-5}
NGB6963	0.077×10^{-5}	0.091×10^{-5}
NGB6975	0.073×10^{-5}	0.081×10^{-5}
NGB7026	0.058×10^{-5}	0.103×10^{-5}
NGB7259	0.047×10^{-5}	0.094×10^{-5}
JHO2001-4	0.079×10^{-5}	0.085×10^{-5}
JHO822	0.068×10^{-5}	0.085×10^{-5}

Discussion

For assessment of salinity tolerance of crops, it is essential that studies continue from the critical germination stage (Wang et al. 2011) through all different growth stages (Zhu et al. 2016). Hence, the present study used both germination under controlled conditions as well as germination and growth under field conditions for screening of the genotypes.

Genotypic differences for salt tolerance were observed in our study and some genotypes displayed tolerance at the germination stage, whereas other genotypes possessed tolerance at the seedling growth stage. Bai et al. (2018) also found 21 out of 248 oats genotypes to be tolerant of both

salinity and alkalinity during germination, with no correlation between tolerances at germination and adult stages or between tolerances of salt and alkali. Verma and Yadava (1986) evaluated 12 varieties of oats for relative tolerance of increasing levels of salinity using combinations of salts similar to those in natural salt-affected soils. Seeds were sown in petri dishes and were exposed to 5 salinity levels (40, 80, 120, 160 and 200 meq salts/L). Germination percentage, root and shoot lengths and dry weight of seedlings decreased with increase in salinity. In general, varieties JHO815, JHO802, JHO816 and UPO201 were found to be more tolerant at germination and seedling stages than other varieties.

Based on the in vitro germination test in our study, the best 5 salinity-tolerant genotypes, which possessed tolerance for both plumule and radicle growth, were: NGB2114, NGB4757, NGB4872, NGB6963 and NGB7252. A few genotypes, e.g. NGB2118-1, NGB4887, NGB6370 and JHO851, showed tolerance in terms of either plumule or radicle growth and moderate tolerance for the other trait. The field-level tolerance study revealed genotypes NGB2120-1, NGB2718, NGB7002, NGB7253, NGB7259 and JHO2001-4 showing salt tolerance during the germination phase. However, of these genotypes NGB7259 was the only one showing survival >50%. These results demonstrate that tolerance of salinity created specifically by NaCl is quite different from natural salinity, where many other salts may be present in the soil and result in more toxic effects.

Cultivation of oats is considered a valuable strategy to utilize saline lands due to its high capacity to accumulate salt ions in its straw, which is widely used as forage for livestock (Han et al. 2013). Therefore, tolerance of abiotic stresses such as salt and drought is a highly important trait in oat breeding. Abiotic stress tolerance is a quantitative trait controlled by multiple genes (Munns and Tester 2008; Deinlein et al. 2014) and identification of lines possessing tolerance of salinity at various growth stages is important for developing salt-tolerant lines in breeding of any crop.

Oats is considered to be a moderately salt-tolerant crop (Grattan 2016). However, it has been rated as having low salt tolerance by Ogle and St. John (2010) and reported to tolerate up to EC4 with an upper limit of EC8, at which it will not even germinate (USDA 1996). The degree of salt tolerance of the crop varies not only with plant species but also with different varieties of the same species (Hernández 2019; Malaviya et al. 2019). Germination and seedling stages have an important bearing on plant development at later stages of growth and ultimately crop yield. Soluble salts in high concentration interfere with a balanced absorption of essential nutritional ions by the plants, resulting in wilting, desiccation and stunted growth.

In the present study, salinity up to EC16 had little effect on germination of any accession. However, in this situation the raised EC was due to NaCl only, and under natural saline sodic conditions responses may be different, as in the field level tolerance experiment only a few genotypes showed germination >50%. The toxic effect of natural saline sodic soil was also apparent in poor germination in the experiment with SSS1 and SSS2 solutions. Earlier reports showed no reduction in yield up to EC3.3, 10% reduction at EC3.6 and 25% reduction in yield at EC4.1 (NSW-DPI 2017). Bai et al. (2018) also found that 68.5 mmol salt/L and 22.5 mmol alkali/L were appropriate concentrations for determining oat tolerance of salinity and alkalinity during germination, whereas Na_2SO_4:NaCl (1:1, 150 mmol/L each) was found optimal for screening oat tolerance of salinity during plant growth and development. For alkalinity tolerance, Na_2CO_3:$NaHCO_3$ (1:1, 75 mmol/L each) was found to be optimal. The field level tolerance experiment indicated that pH level of saline soils is far more important for growth of plants than EC level. Even genotypes showing good germination could not survive after a few weeks. A high NaCl concentration in soil causes a reduction in growth parameters (Sixto et al. 2005) such as fresh and dry weight of leaves, shoots and roots along with a decrease in moisture content (Parvaiz and Riffat 2005). High salinity stress also delays the emergence of nodal roots, leaves and tillers (Córdoba et al. 2001).

Correlation analysis revealed that ion accumulation is positively correlated with biomass and accumulation of Na^+ and Cl^- in straw is negatively ($P<0.05$) correlated with accumulation of K^+ (Wu et al. 2017). Sodium (Na^+) and chloride (Cl^-) are the key ions responsible for both osmotic and ion-specific damage, which significantly reduces crop growth and yield (Munns and Tester 2008). Using comprehensive transcriptome and functional analyses, Wu et al. (2017) showed that salinity stress in oats affects a variety of genes involved in different biological processes, osmotic adjustment and regulatory networks. Bai et al. (2018) found there was no correlation between tolerance of salinity and alkalinity during germination and plant growth stages. Alkalinity mainly decreases chlorophyll concentration, while salinity mainly disrupts water absorption and water balance. With increasing soil salt concentration, production of oats biomass decreases, which coincides with increasing Na^+ and Ca^{2+} concentrations (Zhao et al. 2007).

The finding of higher concentrations of proline in salt-stressed plants than in those growing under Control conditions confirms the usefulness of this biochemical indicator (Ashraf and Harris 2004; Ashraf and Foodlad 2007) and suggests that also in oats this concentration can be used as an indicator for salt tolerance.

Salinity is best characterized by EC of the irrigation water or EC of the saturated soil solution in distilled water. The higher the concentration of dissolved salts, the higher was the EC value. In the field study EC was 3.3, whereas in nature in general, soils with EC≤5 have total dissolved salts of 640 mg/L, while at EC>8 total dissolved salts are above 800 mg/L. In addition, soil in our study was highly alkaline. Few genotypes showed tolerance of both salinity and alkalinity, although genotype NGB7259 was an exception. In an earlier study by Bai et al. (2018), although 3 genotypes proved tolerant of both salt and alkali at both germination and adult stages, tolerance of salinity and alkalinity during germination and plant growth were not correlated. The identification of lines tolerant of different forms of salts as well as at different growth stages provides an opportunity for further gene pyramiding.

Acknowledgments

Authors are thankful to Indian Council of Agricultural Research (ICAR), New Delhi, India for financial support of the work under National Agriculture Technology Project.

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(Note of the editors: All hyperlinks were verified 12 November 2020.)

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(Received for publication 10 January 2020; accepted 1 September 2020; published 31 January 2021)