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'DUS' characterization of an endangered salt tolerant radish landrace (*Newar*)

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In this study, responses to salinity stress of three varieties of radish, *viz.,* 'Newar' (landrace), 'Pusa Mridula' and 'White Excel', were recorded. Additionally, landrace *Newar* was also characterized for 'Distinctness, Uniformity and Stability' (DUS) using 34 descriptors. Results indicated higher salt tolerance in 'Newar' as evidenced by relatively early germination and high early seedling vigour than other varieties regardless of the salinity of the irrigation water. Although salinity stress, especially up to 8.0 dS m^{-1}, had no adverse effect on shoot growth in all the varieties, effects on root growth were quite different. While 'Newar' exhibited non-significant differences in root fresh weight (RFW) at different salinity levels, 'White Excel' displayed nominal variations up to 8.0 dS m^{-1} salinity and 'Pusa Mridula' registered consistent declines in RFW with increasing salinity. 'Newar' plants were found to be efficient in Na^+ exclusion and in maintaining a favourable Na^+ to K^+ ratio in their shoots and roots. Further, proline accumulation was much higher in salt treated *Newar* than in 'White Excel' and 'Pusa Mridula' plants. Based on DUS descriptors, number of leaves, leaf length, and root length and weight were found to be the major distinguishable characters in *Newar*.

Keywords: DUS, Endemic, Ion uptake, *Newar* radish, Plant vigour, Proline, Salinity

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Transition from subsistence farming to commercial agriculture has accelerated agrobiodiversity loss and environmental degradation[1-3]. Adverse impacts of agricultural intensification, viz., loss of traditional ecological knowledge (TEK) and farming practices[3,4], degradation of ecosystem services[5] and decline in biodiversity[6] often drastically reduce agro-ecosystem resilience and productivity, putting the farmers' livelihoods at risk. Despite overall improvements in food availability in the last few decades, widespread hunger and malnutrition still remain significant global concerns; particularly so in the developing countries[7,8] where growing population pressure, pervasive land use and intensive agriculture continue to threaten the agro-ecosystem sustainability[9,10]. Halting the loss of agro-biodiversity is critical to ensuring food and nutritional security; especially in areas where agriculture and related activities are the principal means of livelihood[3,10,11]. Mainstreaming of traditional crops into local diets could also be an effective means of improving the nutritional security and lessening the impact of non-communicable diseases like cardiovascular problems, cancer and diabetes[12,13]. Considering the importance of agro-biodiversity conservation in the face of global environmental change, strategies for arresting the further decline of local crops, landraces and their wild relatives are urgently needed[14,15].

In many areas across the world where high-yielding varieties are either altogether absent or grown on a limited scale[16,17], landraces and farmers' varieties are still widely grown[18]. In India, the advent of the 'Green Revolution' (GR) in the mid-1960s marked a switchover from traditional to modern farming practices, resulting in the gradual loss of local farming systems and associated TEK; particularly in the North western regions comprising Punjab, Haryana and Uttar Pradesh states, where genetically uniform, high-yielding rice and wheat varieties were promoted vigorously[19,20]. Of late, a worrying trend in the loss of traditional crop genetic resources is also becoming evident, even in biodiversity rich and geographically isolated regions like central[21] and eastern[22] India. In such areas, locally adapted crops and landraces are grown in traditional farming systems to meet the diverse nutritional needs of farm families, minimize absolute crop failures caused by extreme weather events, and to ensure higher productivity under marginal situations[21].

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Radish is an important vegetable crop grown world over for its succulent edible roots, which are used as salad, processed into value-added products like pickle and serve as an ingredient in the folk medicine[23,24]. Several farmers' varieties and landraces of radish have been reported from India[25] and other Asian countries[26-28]. A radish landrace, popularly called *Newar* (Syn. 'Jaunpuri Giant') (*Raphanus jaunpurensis* species. *nova*.), endemic to Jaunpur district of Uttar Pradesh, India; has long been valued for promising traits like long crisp roots with a high shelf-life, salt tolerance, use in pickling and seed oil extraction. However, cultivation of this landrace has virtually come to a halt due to changes in land use patterns and consumer preferences, and disappearance of the local seed network[22]. Introduction of high yielding cultivars is known to hasten genetic erosion in radish in other parts of Indian Subcontinent and Central Asia[29]. Since a strong majority of Indian farmers still depend on informal seed networks, the collapse of farmer-to-farmer seed exchange can have a detrimental impact on local agricultural systems[30]. Like other crops, molecular studies have revealed rich genetic variation for important agronomic traits in radish landraces[31,32]. Available evidence suggests that the radish landrace *Newar* performed well under saline conditions in its native habitat: saline water irrigated *Newar* crop produced the best quality roots in terms of length, yield and organoleptic properties compared to those irrigated with normal water[33]. Although some recent investigations have also confirmed salt tolerance in *Newar*[34,35], they did not provide insights into putative mechanisms imparting salt tolerance. Furthermore, salinity levels imposed in these studies were rather low, making it difficult to draw meaningful conclusions.

The Protection of Plant Varieties and Farmers' Rights Act, passed by the Government of India in 2001, is a *sui generis* system for protecting plant varieties and the rights of plant breeders, farmers and village communities. Aimed at the equitable sharing of benefits, the Act defines a 'farmers' variety' as 'a variety traditionally cultivated and evolved by the farmers in their fields' or 'a wild relative or landrace of a variety about which the farmers possess common knowledge'[36]. Generally, farmers' varieties tend to be more homogenous, possess distinct traits and enjoy the consumers' acceptance. In order to be unique and easily distinguishable from others, a farmers' variety should meet the requirements of a DUS test based on morphological and physiological characters called descriptors[37]. Minimal descriptors for DUS testing have also been developed in radish[38].

In this backdrop, a study was conducted with the following objectives: 1. Identification of putative traits imparting salt tolerance to *Newar*; and 2. DUS characterization using selected morphological descriptors.

Research methodology

Study site

Consistent with the research objectives, two separate experiments were conducted. One experiment for working out the salt tolerance of the *Newar* radish landrace was carried out during 2017-18 at ICAR-CSSRI Experimental Farm, Karnal (29°43'N, 76°58'E; 245 m above the mean sea level) in a shade house under natural conditions. Karnal has a semi-arid climate with mean annual rainfall of about 750 mm. Another experiment for DUS characterization was conducted at three different locations including ICAR-CSSRI Experimental Farm, Karnal and at farmers' fields in Karnal and Kaithal districts having similar agro-climatic conditions.

Experimental details

In the first experiment, three radish varieties, viz., *Newar* (landrace), 'Pusa Mridula' (improved public sector variety) and 'White Excel' (private sector hybrid), were evaluated for salt tolerance during germination and early plant growth stages. Seeds were sown in enamel pots containing 20 kg washed sand. Before sowing, seeds were surface-sterilized for 5 min in a 10% sodium hypochlorite solution and then rinsed with distilled water. Ten seeds of each variety were placed at 1 cm depth on November 9, 2017. The bottom of each pot was delved for draining extra water. Salt treatments were imposed one day after sowing using normal tap water control; EC_{iw} 0.5 dS/m and four concentrations of saline water (EC_{iw}: 4, 6, 8 and 10 dS/m in ¼ strength Hoagland solution. Saline solutions were prepared by dissolving the measured quantities of NaCl, $CaCl_2$ and Na_2SO_4 salts, with Na^+: Ca^{2+} and Cl^-: SO_4^- ratios of 4:1, reflecting the ionic composition of saline groundwater in many parts of North-Western India. Salinity was induced by the incremental additions of salts in a step-wise manner up to a week after sowing, to avoid osmotic shock. Thereafter, pots were irrigated every alternate day with 1 L saline solution for maintaining the desired levels of salinity. In another experiment, *Newar* crops were raised at three different locations of Karnal and Kaithal districts of Haryana, India in sodic soils, and the selected morphological descriptors for DUS characterization were recorded.

Data collection

Germination percentage was recorded using the formula: GP (%) = (S/T) ×100; where 'S'= number of seeds germinated and 'T'= total numbers of seeds sown. Percent decrease in germination was used to assess salinity tolerance at the seedling stage. Similarly, 'days to germination' and 'plant vigour' were also recorded by daily visual observations. Different scores (poor: 1, fair: 3, good: 5 and excellent: 7) were assigned to seedlings based on their health and appearance under different salinity treatments. Two-week-old seedlings (n=5) were randomly uprooted from each treatment (in two replicates) for recording the fresh weights of shoots and roots using an electronic balance (SECURA125, Sartorius AG, Gottingen, Germany). Shoots and roots were washed once with the tap water and twice with double distilled water for removing the salt particles and other impurities. Proline concentration in fresh shoot and root samples was determined as described in[39]. Shoot and root samples were dried to a constant weight at 60°C in a hot air oven (NSW, India). Dry tissue (50 mg) was ground and digested in a diacid mixture (10 mL) containing HNO_3 and $HClO_4$ acid (9:4 ratio) using a hot plate digestion system. After proper cooling, the digest was diluted with double distilled water, filtered and final volume was made up to 50 mL. Analysis of Na^+ and K^+ ions was done using inductively coupled plasma emission spectroscopy (ICPE-9000, Shimadzu Europa GmbH, Germany). Distinctness, Uniformity and Stability (DUS) characterization of the radish landrace *Newar* grown at different locations, viz., ICAR-CSSRI, Karnal, Baras village of Karnal district and Sikander Kheri village of Kaithal district (farmer participatory trials), was also done using 32 descriptors developed by ICAR-NBPGR, New Delhi. Different DUS characters were recorded at the specific stages of crop growth when a particular character had the fullest expression[38,39]. Soil saturation paste salinity (EC_e) and pH (pH_s) values revealed the sodic nature of the soils in the DUS testing plots. Soil pH_s at ICAR-CSSRI Experimental Farm, Karnal was ~8.3 and 8.7 at 0-30 and 0-60 cm soil depths, respectively. In contrast, soil EC_e was nearly uniform (~1.0 dS/m) up to 60 cm depth. At the farmers' fields, soil pH_s and EC_e values were ~8.5 and ≤ 1.0 dS/m, respectively, across different soil depths. Sowing was done in the second week of October, 2017 on levelled beds of 3 m x 3 m size. Seeds (n=100) were sown on 25 cm high ridges at 2 cm depth. Row-to-row and plant-to-plant spacings were kept at 45 cm and 30 cm, respectively. Recommended cultural practices for growing a healthy crop were followed.

Data analysis

The experiment was arranged in a factorial randomized block design (RBD) with two replications with 10 seeds/plants per replication. Statistical analyses for different parameters were performed using the SAS 9.3 software [SAS Institute Inc., Cary, USA (licensed version, ICAR-IASRI, New Delhi)].

Results and discussion

Seed germination and early plant vigour

While seed germination was not affected in *Newar* and 'White Excel' up to EC_{iw} of 10.0 dS m^{-1}, 'Pusa Mridula' exhibited only ~93.0% germination at 4.0-6.0 dS/m salinities which further declined to 67.7% and 53.3% at 8.0 and 10.0 dS/m, respectively, reflecting high sensitivity of 'Pusa Mridula' to salt stress during seed germination. Notwithstanding complete seed germination in 'White Excel' even at an EC_{iw} 10.0 dS/m, it invariably took more time than *Newar* for germination at a given salinity; especially above 4.0 dS/m. While *Newar* seeds took an average of 3.5 days for germination at 6.0 to 10.0 dS/m salinity levels, both 'White Excel' and 'Pusa Mridula' varieties averaged ≥5.5 days for germination (Table 1). Irrespective of irrigation water salinity, *Newar* out performed other two varieties in terms of plant vigour. While it had marginally higher plant vigour scores at 4.0 and 6.0 dS/m salinities than the control; virtually no differences in vigour scores in the control and 8.0 dS/m treatments reflected higher salt tolerance in *Newar* (Table 1; Fig. 1). Increasing salinity (0 to 34.4 dS m^{-1}) suppressed the percentage and rate of seed germination in three radish cultivars to varying extents. While 'Antep' was highly tolerant of salinity, 'Beyaz' showed moderate and 'Siyah' low salt tolerance[40]. Increasing soil solution salinity (EC_s 1.0, 2.0, 4.0, 9.0 and 13.0 dS m^{-1}) did not affect the date of seed germination in radish cv. 'Saxa Nova'

Table 1 — Effects of salinity on seed germination and early plant vigour in radish varieties

Genotype	Treatment (EC_{iw}dS/m)	Days to germination	Germination (%)	Plant vigour score
Newar	0.5 (C)	3.0	99.5	6
	4	3.5	99.5	7
	6	4.5	100	7
	8	4.5	99.5	6
	10	4.5	98.5	5
	LSD at 5%	0.90	0.63	0.71
White Excel	0.5 (C)	4.0	99.5	6
	4	5	98.5	5
	6	5.5	98.5	5
	8	5.5	95.0	4
	10	5.5	91.0	3
	LSD at 5%	0.39	3.31	0.78
PusaMridula	0.5 (C)	4.5	97	4
	4	5.5	94.4	4
	6	5.5	94	4
	8	5	66.15	4
	10	6.5	54.55	3
	LSD at 5%	0.90	2.93	1.0

Note: C: Control

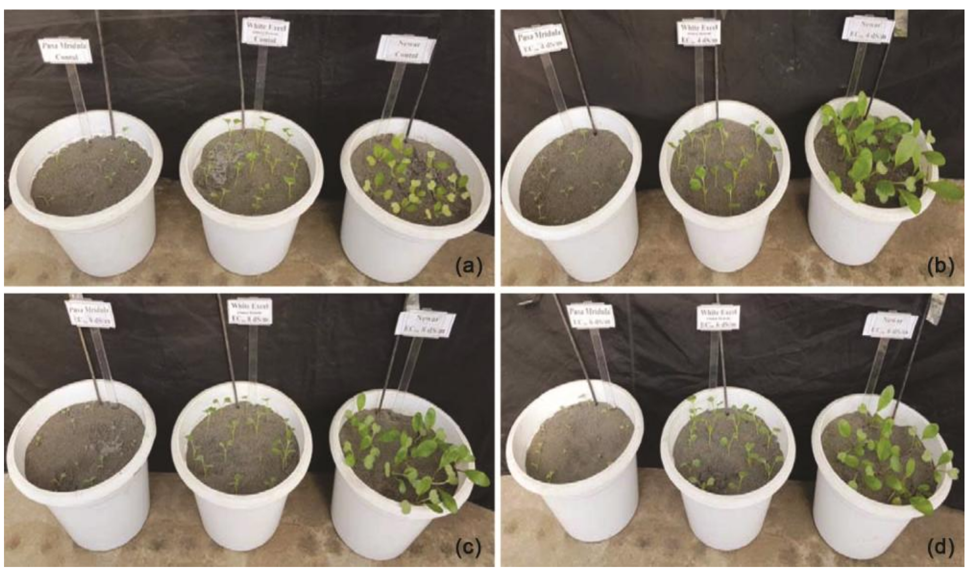

Fig. 1 — Early plant vigour in salt treated radish varieties

appreciably. Interestingly, seeds invariably took fewer days for germination at intermediate salinities compared to both control and the highest salinity treatments[41]. Seed germination declined progressively in radish varieties as salinity increased from 0.7 to 12.0 dS/m; though to a higher extent in the improved variety 'Red Bombay' than in local varieties 'Tasakisan Mula-1' and 'Druti'[42]. Despite better performance than other Brassicaceous crops (cabbage and mustard), NaCl-induced salinity ≥ 8.0 dS/m decreased the percentage and rate of seed germination in radish considerably[43]. These observations suggest that factors like experimental conditions and genetic make-up could greatly influence the response of radish seeds and seedlings to salinity stress.

Shoot and root growth

Shoot fresh weight (SFW) was not affected up to 8.0 dS/m salinity in *Newar* but declined by ~46.0% at 10.0 dS/m compared with the control. A similar trend was noted in 'White Excel' in which SFW also decreased only at the highest salinity. In 'Pusa

Mridula', SFW was slightly higher up to 6.0 dS/m salinity than the control but declined with further increase in salinity. A reverse trend, however, was noted with regard to root fresh weight (RFW) with *Newar* showing non-significant differences at different salinity levels, 'White Excel' exhibiting marginal variations up to 8.0 dS/m salinity and 'Pusa Mridula' displaying consistent declines in RFW with increasing salinity (Table 2). In several crops, shoot growth is more sensitive to salinity stress than root growth[44,45] which has also been corroborated in radish[41]. Japanese wild radishes growing along seacoasts exhibit much higher salt tolerance, and unlike cultivated varieties, are not adversely affected even at exceptionally high salinities[46]. As shown previously, saline irrigation had a growth enhancing effect on *Newar* crop in its native environment[33] and this might explain its better salt tolerance noted in the present study.

Sodium and potassium uptake

Data presented in Table 2 reveal that regardless of the variety, shoot Na^+ was invariably higher in salt-treated than in salt-free radish plants. Nonetheless, the increases in shoot Na^+ at both lower and higher salinities were much larger in 'White Excel' and 'Pusa Mridula' compared to Newar. For example, at 4.0 dS/m salinity, shoot Na^+ was about 27, 90 and 101% higher than the control in Newar, 'White Excel' and 'Pusa Mridula', respectively. Again, at 10.0 dS/m salinity, shoot Na^+ in *Newar* was only about 13% higher than in the control but it was 86 and 142% more in 'White Excel' and 'Pusa Mridula' plants, respectively. Although root Na^+ was also significantly higher in saline treatments, both *Newar* and 'White Excel' plants tended to restrict Na^+ uptake with increasing salinity. In sharp contrast, 'Pusa Mridula' roots displayed an abrupt increase of over 200.0% even at the lowest salinity, suggesting a weaker efficiency for Na^+ exclusion. Increased salinity in the root zone also suppressed K^+ accumulation in shoot and root tissues; albeit in a variety-specific manner. Thus, Newar, in spite of being relatively efficient in Na^+ exclusion, displayed greater reductions in shoot and root K^+ levels than both 'White Excel' and 'Pusa Mridula' at a given salinity. Despite this, salt treated *Newar* plants were able to maintain a favourable Na^+:K^+ ratio in shoots and roots. Compared to respective controls, root Na^+:K^+ ratio was nearly three-fold higher in Newar, five to six times more in 'White Excel' and ten- to fifteen-fold greater in 'Pusa Mridula' at 8.0-10.0 dS/m salinities. Similarly, shoot Na^+:K^+ ratio was nearly five times more in both *Newar* and 'Pusa Mridula' and eight- to ten-fold higher in 'White Excel' at ≥ 8.0 dS/m salinity than salt-free plants (Fig. 2). Like other species, salt treated radish plants usually exhibit increased accumulation of Na^+ and depletion of K^+[47,48]; albeit with strong genotypic differences for ion partitioning in shoot and root tissues. For example, cultivars '40 Days' and 'Desi' displayed the highest leaf and root Na^+ concentrations, respectively, at 160 mM NaCl

Table 2 — Effects of salinity on plant growth and ion uptake in radish varieties

Genotype	Treatment (EC_{iw}dS/m)	SFW (g)	RFW (g)	Shoot Na (ppm)	Root Na (ppm)	Shoot K (ppm)	Root K (ppm)
Newar	0.5 (C)	2.73	0.05	19.67	20.52	86.20	47.27
	4	2.98	0.08	24.90	20.11	49.05	46.37
	6	2.56	0.07	26.04	23.74	25.19	25.45
	8	2.51	0.07	28.56	24.86	25.11	25.68
	10	1.47	0.07	32.35	30.71	28.03	27.06
	LSD at 5%	0.54	0.03	1.27	1.33	4.56	3.89
White Excel	0.5 (C)	9.22	2.33	20.05	25.62	50.53	49.86
	4	11.27	2.53	38.22	27.92	29.76	44.73
	6	10.63	2.72	36.43	33.64	29.44	29.87
	8	9.72	2.40	37.36	34.64	29.36	28.53
	10	7.50	1.33	37.47	34.21	25.94	24.67
	LSD at 5%	0.17	0.01	3.53	3.06	3.14	3.25
Pusa Mridula	0.5 (C)	0.86	0.31	14.07	6.81	33.61	26.58
	4	1.01	0.20	28.31	21.95	24.67	19.57
	6	1.31	0.12	29.84	27.01	20.32	13.33
	8	0.93	0.12	33.94	34.47	20.03	11.23
	10	0.94	0.06	34.15	40.36	18.71	8.65
	LSD at 5%	0.17	0.02	5.73	4.81	4.05	5.29

Note: SFW: Shoot fresh weight, RFW: Root fresh weight compared with the control. Contrarily, the maximum leaf and root K^+ was recorded in 'Lal Pari' and 'Mannu Early' plants, respectively[49]. The observation that Na^+ accumulation was lower in shoots than in roots of salt treated *Newar* plants, which might account for the stability of leaf membranes and better photosynthesis, has previously been reported in radish[48].

Proline accumulation

Salt treated plants accumulate various inorganic and organic osmolytes for maintaining leaf turgor and for creating a gradient for water absorption. Proline is such a major metabolically benign organic solute[50]. Salt treatment enhanced the proline accumulation in shoots of all the radish varieties, though to a much greater extent in Newar, which showed an increase of about five times at moderate salinities (6.0-8.0 dS/m) and nearly fourteen-fold higher shoot proline at 10.0 dS/m salinity than the control. In comparison, shoot proline was only about three to three and half times more in both 'White Excel' and 'Pusa Mridula' plants at 8.0 and 10.0 dS/m salinity levels, respectively (Fig. 3). Salt treated radish plants displayed considerably higher leaf and root proline concentrations than controls regardless of the growth stage[51]. Although NaCl (80.0 or 160.0 mM) application significantly increased leaf proline in radish cultivars, 'Mannu Early' displayed the highest proline accumulation reflecting that proline enhances the plant salt tolerance in a genotype-dependent manner[52]. Exogenously applied proline is known to alleviate the adverse effects of salinity on important physiological processes in radish[53].

DUS description

For DUS (Distinctness, Uniformity and Stability) characterization, *Newar* crop was sown at three locations of Karnal and Kaithal districts of Haryana, India in a replicated trial with 3 replications and 100 plants per replication[54] (Fig. 4). Row-to-row and plant-to-plant distances (45 cm and 30 cm, respectively) were kept higher than commonly

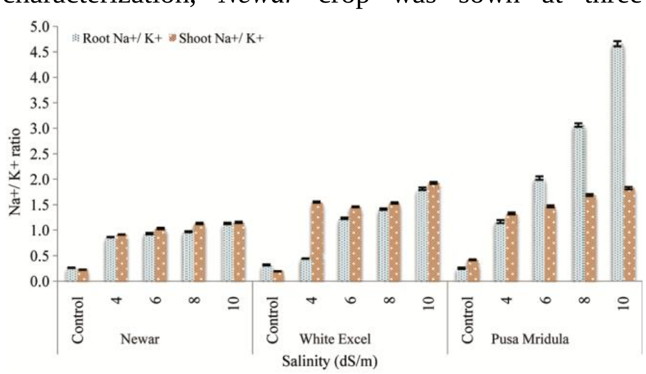

Fig. 2 — Sodium and potassium ratio in leaves and roots in salt treated radish varieties. The vertical bar indicates LSD at 5% level

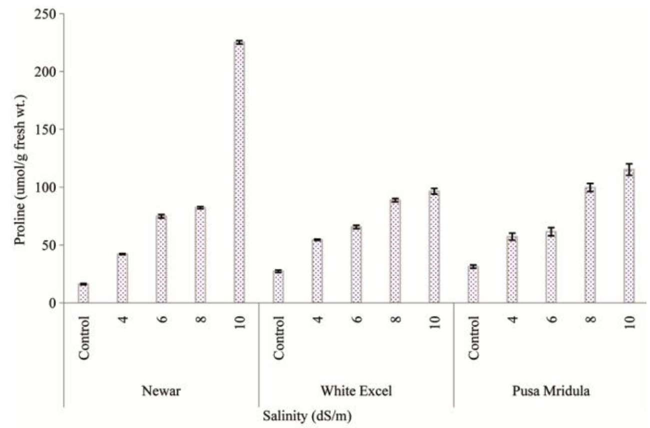

Fig. 3 — Leaf proline accumulation in salt treated radish varieties. The vertical bar indicates LSD at 5% level

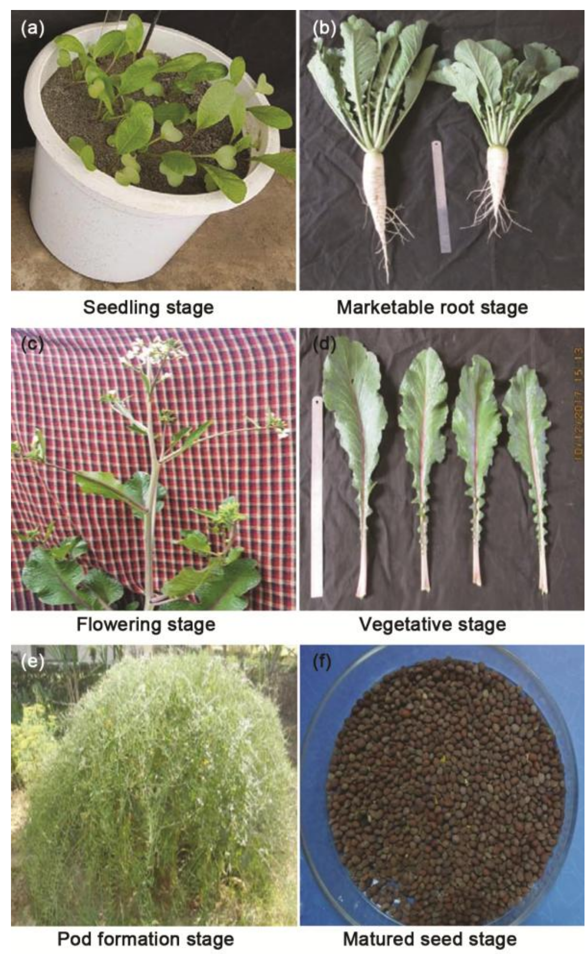

Fig. 4 — A view of the DUS characterization of radish landrace *Newar* landrace in Karnal (Haryana), India

followed in India[55,56]; apparently because *Newar* plants grow profusely and require relatively more space for completing their life-cycle. A total of 32 quantitative and qualitative descriptors were used at different stages of crop growth, *viz*., 30 days after sowing, flowering, marketable root harvest stage and harvesting for DUS characterization. A perusal of the observations presented in Table 3 revealed considerable differences growth habit and root traits in *Newar* than previously reported for radish germplasm in India[55-58]. Specifically, number of leaves per plant, leaf length, root length and weight, and 1000 seed weight seemed to be the most distinguishable characters in *Newar*.

Conclusion and future prospects

Despite considerable salt tolerance, radish landrace *Newar* has remained neglected and under researched. This study provides evidence that *Newar* could be a source of potentially novel genes for improving the salt tolerance of radish and related Brassicaceous crops, especially with the aid of marker-assisted breeding. As this landrace is virtually on the verge of extinction, concerted efforts are needed for introducing it as a viable commercial root crop in salt-affected areas. Convincing evidence on income generating potential of *Newar* cultivation is currently lacking which seems to be a prerequisite to increasing its adoption as a commercial crop in

Table 3 — Characterization of radish landrace *Newar* based on DUS descriptors

S N.	Characteristic	Stage of observation	Remark
1	Early plant vigour	30 DAS	(7) Good
2	Plant growth habit	Flowering	(6) Elongate branching stem supporting leafs and/or heads
3	Leaf colour	30 DAS	(4) Dark green with purple midribs
4	Leaf length (cm)	MRHS	39.5 ± 1.0
5	Leaf width (cm)	MRHS	11.5 ± 1.0
6	Leaf margin	MRHS	(1) Crenate
7	Leaf apex shape	MRHS	(4) Oval
8	Leaves per plant	MRHS	25.0 ± 5.0
9	Leaf pubescence	MRHS	(7) Abundant
10	Petiole length (cm)	MRHS	7.5 ± 1.5
11	Petiole colour	MRHS	(7) Purple
12	Days to 50% root harvest	MRHS	50.0 ± 3.0
13	Crown head habit	MRHS	(1) Erect
14	Crown head colour	MRHS	(1) Light green
15	Crown head diameter (cm)	MRHS	5.0 ± 1.5
16	Root length (cm)	MRHS	35.0 ± 3.5
17	Root diameter (cm)	MRHS	4.7 ± 0.5
18	Root Branching	MRHS	(3) Present
19	Root skin colour	MRHS	(2) Creamy white
20	Root shape	MRHS	(2) Triangular
21	Root tail	MRHS	(1) Acute
22	Root weight (g)	MRHS	315.0 ± 25.0
23	Root pithiness	MRHS	(0) Absent
24	Root pungency	MRHS	(3) Mild
25	Root flesh texture	MRHS	(1) Crisp
26	Bolting habit	Flowering	(1) Tropical
27	Inflorescence type	Flowering	(1) Single raceme
28	Days to 50% flowering	Flowering	85.0 ± 3.0
29	Flower head size	Flowering	Average number of effective tillers: 8; with flower diameter of 2.5 cm
30	Seed coat colour	Harvesting	(3) Light brown
31	Weight of pod per plant (g)	Harvesting	240.62
32	Seed weight per plant (g)	Harvesting	60.90
33	1000 seed weight (g)	Harvesting	13.45
34	Biotic stress susceptibility	Throughout crop season	(1) Very low or no visible sign of susceptibility

Note: DAS- Days after sowing; MRHS- Marketable root harvest stage salt-affected soils. Investigations are needed to establish the commercial and health-promoting potential of the edible *Newar* seed oil. Preliminary results from the farmer participatory trials of this variety are encouraging and efforts are underway for its evaluation and possible commercialization in other saline/sodic parts of the country.

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