



Screening lentil (*Lens culinaris*) RIL population for high yield and aluminium toxicity tolerance under acidic field and hydroponic conditions

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Received: 19 January 2023; Accepted: 27 March 2024

ABSTRACT

The present study was carried out during winter (*rabi*) season of 2020–21 at two locations namely the experimental field of College of Post Graduate Studies in Agricultural Sciences (Central Agricultural University, Imphal, Manipur), Umiam, Meghalaya and Agro-forestry experimental plot of ICAR-North-Eastern Hill Region, Meghalaya followed by hydroponics screening during winter (*rabi*) season of 2021–22. The experiments involved screening of a recombinant inbred line (RIL) population of lentil (*Lens culinaris* Medik) obtained from a cross between BM-4 (Al sensitive parent) and L-4602 (Al tolerant parent) for high yield and Al (aluminium) toxicity tolerance through evaluation in the acidic field conditions, character association studies and root growth studies under hydroponics having toxic Al concentration of 148 μ M. Highly significant variance due to genotypes revealed presence of sufficient variability for all the traits except number of seeds/pod (SP). Among the characters, high H_{bs}^2 associated with high GA% were recorded in number of primary branches/plant (NB), plant height (PH) and 100-seed weight (SW). Path analysis revealed that, number of pods/plant (PP) (0.840) had the greatest direct effects in influencing seed yield/plant (SYPP), followed by biological yield/plant (BYPP) (0.795), number of seeds/pod (SP) (0.474), number of primary branches/plant (NB) (0.309) and harvest index (HI) (0.307). Correlation studies among root and shoot parameters under hydroponic studies revealed significant correlation between root dry weight (RDW) and shoot dry weight (SDW); shoot fresh weight (SFW) and root fresh weight (RFW); total root length (TRL) and surface area (SA); and haematoxylin stain score (STNS) and root regrowth (RRG). Based on mean performance of SYPP and attributing traits, combined with root growth studies under hydroponics, the high yielding and Al toxicity tolerant genotypes identified were LRIL-37, LRIL-22, LRIL-68, LRIL-96 and LRIL-97. In addition to serving as parents in hybridization programmes, these genotypes may undergo additional evaluation in multiple environments prior to final release in an effort to enhance performance.

Keywords: Aluminium toxicity, High yield, Hydroponics, Lentil, Tolerance

Lentil (*Lens culinaris* Medik) popularly known as “Masoor” in India, is a predominant pulse crop that is utilized for feed and food due to its high protein content (20.6–31.4%) in the grains and straw (Urbano *et al.* 2007). Through their unique property of fixing atmospheric nitrogen, legumes such as lentil uplifts soil fertility and the activity of ecosystems, thus forming an important part of various cropping systems (Peoples *et al.* 2009). Lentil, India's third-most significant pulse crop was grown on 1.32 million hectares of land in 2019–20, yielding 1.18 million tonnes of yearly production and 894 kg/ha of productivity

(Anonymous 2020). However, the production was not sufficient to meet the domestic demands which led to an import of additional 1.11 million tonnes of lentil during 2021–22 (Anonymous 2022).

Because of its favourable climate, Meghalaya has enormous possibility to expand the area used for lentil cultivation. However, the vast majority of Meghalaya's soils (2.24 million hectares) are acidic (Majumdar *et al.* 2022), where aluminium (Al) toxicity becomes the major threat to crop production, particularly below pH of 5.0, where it transforms into phytotoxic forms causing inhibition of root growth, decrease in plant vigour and lower grain yields (Kochian *et al.* 2005). Among various Al toxicity symptoms, rapid root growth inhibition, is the first visible symptom (Kochian *et al.* 2005), widely used as a biomarker to determine plant's sensitivity to Al, specially under hydroponics systems (Singh *et al.* 2016).

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Among various screening techniques for Al tolerance, haematoxylin staining and root regrowth studies under hydroponics in conjugation with field evaluation provide better accuracy in screening and identification of Al toxicity tolerant genotypes (Singh and Raju 2011, Singh *et al.* 2012, Singh *et al.* 2018).

It is also important to identify traits in lentil that results in higher yields under acidic field conditions to facilitate better selection criterion. Therefore, the current study was aimed for screening Al stress tolerance in lentil RILs to ascertain agronomical traits contributing more to yield in acidic field conditions, and identify the high yielding Al tolerant RILs based on field evaluation and hydroponics assay.

MATERIALS AND METHODS

Experimental material: The materials used in the experiment included 150 F_6 RILs of lentil (Supplementary Table 1), the two parents, viz. BM-4 (sensitive to Al) and L-4602 (tolerant to Al) and two checks namely DPL-62 and PDL-1 obtained from ICAR-Indian Agricultural Research Institute, New Delhi. Generation advancement of the lentil F_3 population (obtained from ICAR-Indian Agricultural Research Institute, New Delhi) was done following single seed descent method till F_6 generation to obtain homozygosity in the RILs, followed by final evaluation for Al toxicity tolerance.

Evaluation of lentil genotypes under acidic field conditions: The present study was carried out during winter (*rabi*) season of 2020–21 at the two locations namely, the experimental field of College of Post Graduate Studies in Agricultural Sciences (Central Agricultural University, Imphal, Manipur), Umiam Meghalaya (soil $pH=5.09$) and ICAR-North Eastern Hill Region, Meghalaya (soil $pH=4.48$). Both these fields displayed acidic soil reaction with the issue of Al toxicity. The soil properties and nutrient status of the corresponding experimental plots are included in Supplementary Table 2. The experiment was laid out in randomized block design (RBD) with 3 replications at both the locations following standard agronomic practices. The observations were recorded for days to 50% flowering (DF),



Fig. 1 Setup for screening lentil genotypes for Al toxicity tolerance using hydroponics culture.

days to maturity (DTM), plant height (PH, cm), number of primary branches/plant (NB), number of pods/plant (PP), number of seeds/pod (SP), seed yield/plant (SYPP, g), 100-seed weight (SW, g), biological yield/plant (BYPP, g) and harvest index (HI, %).

Screening of lentil genotypes under hydroponics culture: The genotypes were screened under hydroponics culture in the Plant Breeding lab of College of Post Graduate Studies in Agricultural Sciences (Central Agricultural University, Imphal, Manipur), Umiam Meghalaya during *rabi*, 2021–22. The haematoxylin staining method was used to differentiate the lentil genotypes cultured under hydroponics (Fig. 1) as tolerant and sensitive based on the scores of staining intensities as per Polle *et al.* (1978) and Singh *et al.* (2018), viz. 0, No staining (means tolerant); 1, Partial staining (means moderately tolerant); 2, Moderate staining (means moderately sensitive); 3, Complete staining (means sensitive). Root re-growth after staining (Fig. 2) based on the classification suggested by Singh *et al.* (2012, 2018) is a reliable parameter to distinguish the genotypes for their Al toxicity tolerance. Root regrowth studies after haematoxylin staining under hydroponics culture was performed by following the protocol of Singh *et al.* (2018). Root re-growth after staining was scored as Sensitive (<0.5 cm); Moderately tolerant (0.5–1 cm); and Tolerant (>1.0 cm).

Statistical analysis: Pooled data obtained for the studied traits were utilized for ANOVA, estimation of genetic

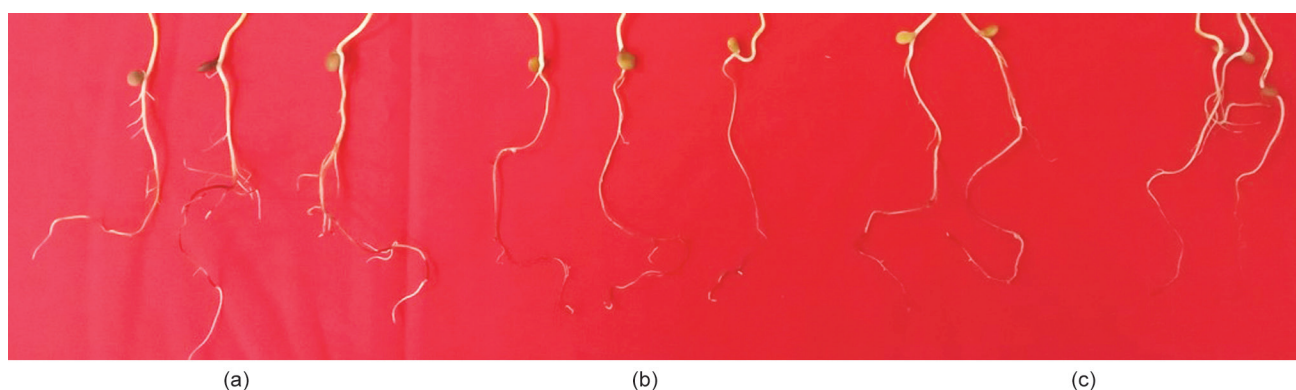


Fig. 2 Genotypes identified as (a) tolerant; (b) moderately tolerant; and (c) sensitive based on root re-growth measurements 48 h after haematoxylin staining.

parameters, correlation and path analysis. Root growth data under hydroponics were subjected to correlation study following Singh and Choudhary (1985).

RESULTS AND DISCUSSION

The variance analysis when aggregated over two sites (Table 1) showed that with the exception of PP, genotypic variance was significant for the studied traits suggesting the presence of ample amount of variability in the genotypes, and the high scope of selection for these traits under acidic soil conditions. Highly significant genotype $\times$ location interaction observed for the traits SYPP, 100-SW, PP, D50F, PH, BYPP and HI suggested substantial interplay between these genotypes and the specific environments of the two locations for the studied characters. These findings were consistent with earlier research on lentils (Dugassa *et al.* 2014, Pant *et al.* 2019, Abbas *et al.* 2019, Verma *et al.* 2022).

Number of pods/plant displayed the highest genotypic coefficient of variation (27.46%), followed by SYPP (26.15%), BYPP (20.52%), and PB (20.51%) (Supplementary Table 3). The traits with the highest PCV were HI (41.17%), PP (39.89%), SYPP (36.20), BYPP (28.66%), and NB. These results show that environmental factors like soil acidity,

have a significant influence on these traits' phenotypic expression. Heritability measures the amount of phenotypic variation that is contributed by genetic causes. In our study, heritability was highest for DTM (90.18%) followed by PH (86.82%), NB (81.52%), DF, 100-SW and PP. High heritability associated with high GA% was recorded for NB, PH and 100-SW for which additive genes most likely had a greater impact, suggesting the efficiency of phenotypic selection favouring these characters, whereas non-additive genes most likely contributed to the inheritance of the other traits. While Dugassa *et al.* (2014) recorded higher values of heritability associated with high GA% for PP and biological yield in lentil, Sharma *et al.* (2022) reported high GCV and PCV along with high heritability and GA% for 100-SW, seed yield, and biological yield. Chowdhury *et al.* (2019) observed presence of high heritability with high GA% for number of seeds/plant followed by number of pods/plant in lentil. Moderate to high measures of heritability, GCV, PCV and GA% were reported for number of secondary branches, pods/plant and seeds/plant (Satpathy and Debnath 2020). The best genotypes identified for acidic field circumstances were LRIL-18, LRIL-22, LRIL-37, LRIL-59, LRIL-63, LRIL-68, LRIL-75, LRIL-80, LRIL-92, LRIL-96, and LRIL-97,

Table 1 Pooled analysis of variance for agronomic traits in 154 lentil genotypes grown in two locations

Source	Degrees of freedom	DF	DTM	PH (cm)	NB	PP	SP	100-SW (g)	SYPP (g)	BYPP (g)	HI (%)
Replications/Block	4	53.22	48.4	11.17	2.45	1886.54	0.032	5.34	0.84	1.024	0.003
Treatments	153	221.38**	126.24**	92.80**	6.79**	3332.40**	0.067	1.63**	6.67**	14.20**	0.164**
Environments	1	14260.71**	9527.15**	6602.06**	1631.24**	50297.80**	0.034	0.82	404.99**	1277.10**	0.004
T $\times$ E	153	24.04**	0.94	3.07**	0.12	942.48**	0.006	0.14**	1.76**	3.66**	0.12**

**, 1% level of significance; *, 5% level of significance.

DF, Days to 50% flowering; DTM, Days to maturity; PH, Plant height (cm); NB, Number of primary branches; PP, Number of pods/plant; SP, Number of seeds/pod; 100 SW, 100 seed weight (g); SYPP, Seed yield/plant (g); BYPP, Biological yield/plant (g); HI, Harvest index (%)

Table 2 Path coefficient showing direct and indirect effects of 9 quantitative traits towards seed yield for 154 lentil genotypes grown in two locations

	DF	DTM	PH	NB	PP	SP	100SW	BYPP	HI	SYPP
DF	0.074	-0.015	-0.012	0.002	0.055	0.001	0.028	0.022	-0.009	0.001
DTM	0.000	0.038	-0.011	0.002	0.044	0.000	0.033	0.011	-0.005	-0.036
PH	0.000	-0.008	0.140	0.006	0.126	0.002	0.026	0.032	-0.002	-0.044
NB	0.000	-0.002	-0.009	0.309	0.208	0.001	0.030	0.052	-0.003	0.032
PP	0.000	-0.001	-0.006	0.007	0.840	0.005	-0.144	0.082	0.021	0.878
SP	0.000	-0.000	-0.011	0.005	0.375	0.474	0.026	0.055	0.011	0.010
100SW	0.000	-0.002	-0.002	0.002	-0.279	0.000	0.195	0.022	0.000	0.454
BYPP	0.000	-0.003	-0.011	0.014	0.593	0.005	0.085	0.795	-0.009	0.121
HI	-0.000	0.002	0.001	-0.001	0.240	0.002	0.000	-0.014	0.307	0.078

Coefficient of determination: 0.955; Effect of the residual variable: 0.211.

DF, Days to 50% flowering; DTM, Days to maturity; PH, Plant height (cm); NB, Number of primary branches; PP, Number of pods/plant; SP, Number of seeds/pod; 100 SW, 100 seed weight (g); SYPP, Seed yield/plant (g); BYPP, Biological yield/plant (g); HI, Harvest index (%).

based on seed yield and ascribed character performance (Supplementary Table 4).

The environment has a significant impact on yield, contributing to its complex nature. Thus, directly selecting seed yield without considering other attributing traits may be ambiguous. Thus, for selecting yield and its yield attributing traits, path coefficient analysis was considered as a more successful strategy (Mahajan *et al.* 2011). In the present study (Table 2), PP (0.8408) had the greatest direct effects in influencing SYPP, followed by BYPP (0.7955), SP (0.4745), NB (0.3095) and HI (0.3073) which were the major and most important traits contributing to seed yield and selecting such traits will contribute to maximum increase in seed yield.

BYPP, SP, HI, NB and PH had the highest positive indirect effects on SYPP through PP. Thus, these traits are major parameters contributing to seed yield and should be simultaneously considered for improving seed yield. Sharma *et al.* (2022) observed that biological yield, number of primary branches, harvest index, and number of pods per plant displayed highest direct effect on seed yield in lentil. The residual effect was estimated to be 0.2110 which indicated that about 21.10% of the variation in yield was due to unknown factors which if considered in this experiment would have completely explained the yield variation. Similar findings were also reported by Singh and Srivastava (2013), Chowdhury *et al.* (2019), Hassan *et al.* (2021) and Takele *et al.* (2022) in lentil.

Haematoxylin staining: Haematoxylin staining of the primary roots of seedlings treated with Al is an important criterion to differentiate the genotypes as tolerant or sensitive based on their staining intensity. The Al sensitive parent BM-4 had a score of 3.00 that corresponds to deep staining, while the Al tolerant parent L-4602 has a score of 2.33 corresponding to moderate staining. In the RILs the staining scores ranged from 1.67–3.00 which corresponded to medium to dark staining, while not one of the RILs exhibited complete lack of staining (Supplementary

Table 5). Previous studies in lentil by Singh *et al.* (2012) reported absence of notable variation for haematoxylin staining between the genotypes and the staining scores that corresponded to moderate to deep staining. Studies on chickpea by Singh and Raje (2011) suggested that the tolerant parents ICC-14880 and IPC92-39 developed none or partial haematoxylin staining, demonstrating a high level of aluminium stress tolerance. Singh and Choudhary (2010) reported that in pea the parents Azad P1 and PC-55-11-1-2 displayed partial staining, exhibiting high degree of Al toxicity tolerance in contrast to Al-sensitive genotypes which developed intense staining. Similarly, haematoxylin staining was used successfully to differentiate genotypes for resistance to Al stress in several crop species (De Macedo *et al.* 2009, Ma *et al.* 2018, Abate *et al.* 2022).

Root regrowth after haematoxylin staining: Staining of roots of lentil genotypes was further aided by root regrowth studies to differentiate the lentil genotypes based on their innate potential to recover from Al stress. When exposed to Al stress, tolerant genotypes show a greater rate of primary root regrowth than sensitive genotypes. Singh *et al.* (2016) revealed that in lentil genotypes, root regrowth ranged from 1.20–1.60 cm in resistant genotypes, while it varied from 0–0.47 cm in sensitive cultigens. Moderately resistant genotypes displayed a range of 0.43–1.00 cm. In the current study, RRG in the genotypes ranged from 0.13–2.38 cm and 54 lentil RILs showed tolerance response based on RRG estimates out of which genotypes identified with highest root regrowth were LRIL-97, LRIL-136, LRIL-48, LRIL-37, LRIL-130, LRIL-143, LRIL-148, LRIL-116, LRIL-125 and LRIL-139. These genotypes also corresponded to lower haematoxylin stain scores (Supplementary Table 5). Similarly, in chickpea genotypes evaluated for root regrowth, it was observed that tolerant parents exhibited longer root regrowth (3.45 and 2.58 cm) than sensitive parents (0.44 and 0.41 cm) (Singh and Raje 2011). Singh and Choudhary (2010) studied root regrowth in pea, where Al toxicity tolerant parents showed significantly

Table 3 Correlation between root and shoot characteristics in hydroponics study

	SL	RFW	SFW	RDW	SDW	TRL	SA	RV	STNS	RRG
RL	0.46**	0.089	0.0555	0.1153	0.0496	0.44**	0.37**	0.1452	-0.0605	0.0665
SL		0.1271	0.0892	0.1	0.0363	0.39**	0.36**	0.21**	-0.0578	0.077
RFW			0.97**	0.42**	0.43**	0.0404	0.0007	0.0219	-0.0065	0.0482
SFW				0.39**	0.44**	0.0003	-0.0105	0.0301	-0.0062	0.0731
RDW					0.87**	0.0478	0.0513	0.0411	0.0515	0.0281
SDW						-0.0153	0.0325	0.058	0.1208	0.0476
TRL							0.81**	0.53**	0.0026	-0.1001
SA								0.83**	-0.0253	-0.0588
RV									-0.0844	0.009
STNS										-0.06**

** = 1 % level of significance; * = 5 % level of significance.

RL, Root length; SL, Shoot length; RFW, Root fresh weight; SFW, Shoot fresh weight; RDW, Root dry weight; SDW, Shoot dry weight; TRL, Total root length; SA, Surface area; RV, Root volume; STNS, Haematoxylin stain score; RRG, Root regrowth.

longer root regrowth as compared with sensitive parents. These results are in agreement with Singh *et al.* (2015), Silva *et al.* (2010) and Stodart *et al.* (2007). Based on the performance of RILs under acidic field condition in terms of higher yield potential and tolerance response to toxic levels of Al concentration under hydroponics, the desirable RILs identified were LRIL-37, LRIL-22, LRIL-68, LRIL-96 and LRIL-97 (Supplementary Table 6).

Correlation study: Significant correlation was found between root dry weight (RDW) and shoot dry weight (SDW) based on a simple correlation study between root and shoot characters under hydroponic study (Table 3). There was a significant correlation between shoot fresh weight (SFW) and root fresh weight (RFW). Total root length (TRL) was highly correlated with surface area (SA) in hydroponics. Haematoxylin stain score (STNS) was negatively and significantly correlated with root regrowth (RRG), suggesting that as stain intensity increases, RRG decreases. However, RRG under hydroponics was significantly correlated to seed yield/plant in the acidic field.

Thus, it can be concluded that the genotypes identified as high yielding and tolerant from our field study and hydroponics analysis are potential genotypes that can be further screened and released as superior yielding varieties tolerant to Al toxicity and suitable for acidic soils of Meghalaya or can be used in various hybridization programmes as parents or donors of Al toxicity tolerant loci. Significant correlation among root and shoot parameters under hydroponic study has been observed. PP, BYPP, SP, NB and HI are the major traits contributing to seed yield and selecting such traits will result in maximum enhancement in seed yield. Further studies are required to determine the tolerance mechanisms contributing to Al toxicity stress tolerance in pulses like lentil.

ACKNOWLEDGEMENT

Authors are grateful to DST-INSPIRE for financial support to the scholar in terms of fellowships and contingency.

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