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Research article

Comparative stress physiological analysis of aluminium stress tolerance of indigenous maize (*Zea mays* L.) cultivars of eastern Himalaya

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ABSTRACT

Yield potential of maize having distinct genetic diversity in Eastern Himalayan Region (EHR) hill ecologies is often limited by Al toxicity caused due to soil acidity. Stress physiological analysis of local check exposed to 0–300 μM Al under sand culture revealed that 150 μM Al as critical and 200 μM Al as tolerable limit. Increase in Al from 0 to 300 μM reduced total chlorophyll, carotenoids by 74.8 % and 44.7 % respectively and enhanced anthocyanin by 35.3 % whereas LA, SLW and SL have reduced by 81.3%, 21.3 % and 47.8 % respectively. R/S ratio was 51.0 and 13.7 % higher at lower Al levels (50 μM and 100 μM) and photosynthetic, transpiration rate and TDM were 62.5 %, 42.9 % and 78.6 % lower at higher Al (300 μM) as compared to control. TRL, RSA, RDW and RV at higher Al (300 μM) were 92.6 %, 98.7 %, 78.7 and 97.5 % lower over control respectively. Root and shoot Al and PUpE at higher Al (300 μM) was 194.0, 69.2 and 830 % higher whereas PUE decreased to 88.5 % over control. Evaluation of 31 indigenous maize cultivars at 0, 150, and 250 μM Al in sand culture, alongside tolerance scoring and assessment, revealed that Megha-9, Megha-10, and MZM-19 exhibits high Al tolerance, Megha-1, MZM-22, and MZM-42 demonstrated moderate tolerance, whereas Uruapara, Sublgarh, and BRL Para were identified as Al-sensitive. Stress physiological parameters like SDW, TDM, TRL, SL and LA contributed 46.02 % of variability to PC1, whereas A, RV, RSA, anthocyanin and Chlorophyll_b, contributed 13.56 % of variability to PC2. Highest values of CMS, SL, LP, LA, TRL and anthocyanin were recorded in cluster I having sensitive cultivars while highest CMS, SL, LA, LP, TRL and RSA were found in cluster II having moderately tolerant cultivars and highest mean values for TRL, RSA, LP, LA, CMS and SL were recorded in cluster III having highly Al stress tolerant cultivars. The traits viz., A, RV, RSA, anthocyanin and Chlorophyll_b, total chlorophyll and TDM were emanated as physio-morphological for assessing Al toxicity stress tolerance in Maize with

Abbreviations: A, Assimilation rate; Al, Aluminium; LA, Leaf area; P, Lipid peroxidation; SLW, Specific leaf weight; SL, Shoot length; R/S ratio, Root to shoot ratio; TDM, Total dry matter; TRL, Total root length; RSA, Root Surface area; RDW, Root dry weight; RV, Root volume; PUpE, Phosphorus uptake efficiency; PUE, Phosphorus utilization efficiency.

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high divergence values. Tolerant cultivars showing 63.4 % and 22.4 % higher anthocyanin at 150 μM Al and 250 μM Al than moderately tolerant one in acid soil experiment with increased root Al, shoot Al, root P and shoot P by 42.6 %, 11 %, 95.1 % and 34 % respectively were emerged as promising for novel maize improvement under acid soils of EHR.

1. Introduction

Soil acidity is one of major edaphic factors that impacts productivity of approximately 70 % of arable land around the world and about one-third of the cultivated land in India [1]. Majority of these soils in India are concentrated in fragile ecosystems of Eastern Himalaya Region (EHR) with 65 % of its arable area having pH below 5.5 and > 80 % of total geographical area (≈23 Mha) is occupied by acid soils having pH < 6.5 [2,3]. Crop productivity on such soils is severely constrained by aluminum (Al) and iron (Fe) toxicity, phosphorus (P) deficiency, low base saturation, impaired root activity and crop nutrition [4,5]. As levels of soil acidity along with its associated impacts on soil fertility and crop productivity are expected to further intensify under climate change in EHR [6–8], the efforts to manage soil acidity are invariable for enhanced food security in the region.

In acidic soil harmless Al in soil solubilised into phytotoxic trivalent (Al^{3+}) cation which impair and restrict root growth and architecture that is important for robust crop health and productivity [4]. Root apex or the distal transition zone (DTZ) in particular is the specific site of Al stress sensitivity [9]. Various investigation established that the stunted root growth and reduced root activity is caused by alterations of root cell modulation and root development due to Al [10,11]. Most evident symptom of Al toxicity is simultaneous induction of β-1,3-glucan (callose) synthesis which can be detected even at micro molar concentrations of Al [12,13]. Due to strong affinity of Al towards the cell wall of root epidermal and cortical cells in roots, highest injuries caused by Al toxicity to cell wall, plasma membrane surface, cytoskeleton and nucleus [14]. Rapid accumulation of Al in the cell wall, apoplast of the root apex, plasma membrane as well as symplasm affects many processes of root growth [13]. Plasma membrane is negatively charged surface, rich in phospholipids, representing sensitive target of the Al phytotoxicity [15]. Al can also affect root metabolism, new cell formation and elongation in the extension region of the root leading to significant reduction in lateral root size [9]. However, plant responses to Al toxicity vary with the crop species [13]. Also, to counter the toxic impacts of Al; plants secrete low molecular weight organic acids (LMWOs) from intact plant roots [16,17].

Apart from P fixation in acidic soil due to ferric oxide and alumina, P use efficiency is also affected by Al toxicity owing to insoluble Al–P compound formation despite high P content in soil [18]. Few research studies have showed that P nutrition play a positive role in alleviating Al toxicity in plants such as wheat [19], buckwheat [20], and sorghum [21]. However, there is a dearth of information regarding the holistic mechanisms of Al-induced inhibition of root proliferation and underlying mechanisms of Al and P nteractions, specific plant processes, stage of crop for setting the critical limits of Al toxicity.

In India, maize is the third most important food and cash crop and it is extensively cultivated across different seasons in most of the states for multiple purposes including grain, feed, fodder, green cobs, sweet corn, baby corn and popcorn. In the EHR, it is majorly cultivated by hill farmers during rainy season (April to September) and its productivity is greatly affected by soil acidity having pH < 5.5 [7]. As vast stretches of mountain soils (≈5.6 Mha area) having pH < 4.5 are currently present in the EHR and accordingly yield performance of maize crop are reported to be suboptimal (<1.5 t/ha) as compared to the national average (2.5 t/ha). As use of lime and other amelioration materials are not feasible in hill slopes; there is an urgent need to understand the apparent adaptive mechanism in these soils so that yield losses can be minimized. One of the major and congenial approaches to enhance crop productivity acidic soil is to screen and identify Al tolerant genotypes among indigenous crop cultivars for EHR [22]. Several screening methods for Al tolerance such as solution, sand and soil cultures, root re-growth and hematoxylin staining techniques and field screening were explored and validated by previous researchers [23–26]. However, reliable ranking of tolerance in the field screening is becoming difficult due to temporal and spatial variation coupled with being cost intensive and time consuming nature of evaluation [27]. Since Al toxicity mostly effects root elongation so as to observe impact of Al toxicity most of the work on Al tolerance and screening of genotypes has been conducted using nutrient solution techniques [23–26]. Keeping ample scope of maize cultivation with wider genetic stock in EHR in view, the present study is aimed at systematic characterization of Al stress response under graded dose of Al using key stress indicators to determine the critical and tolerable levels of Al. This study also attempted to identify differential Al tolerant maize lines with designated stress responsive physio-morphological attributes with special emphasis on extent of growth impairment in root and shoot level, Al partitioning and P uptake pattern for enhanced maize productivity in EHR.

2. Materials and methods

2.1. Plant material

For Al gradient stress characterization, moderately Al tolerant local maize cultivar viz., RCM-1-76 that was developed by ICAR Research Complex for NEH Region, Umiam for acid soils of North Eastern Hill Region (NEHR) was taken as check variety. For screening and identification of differential Al toxicity tolerance, thirty (30) maize cultivars were collected from different districts of the seven states of NEHR of India. Details of the cultivars along with place of its collection are given in Supplementary Table 1.

2.2. Microcosm sand & soil culture experiments

The trial was conducted by using two different growth medium (i) Sand and (ii) soil experiment for a period of 40–45 days.

2.2.1. Sand culture experiment

Sand culture experiment was carried out in Plant growth chamber (PGW40, Conviron®, Canada) with optimal growth conditions of 16/8h photoperiod at 600 μE/cm^2/s, day/night temperature 25–28/15-20 °C, and 70 % relative humidity (RH). Circular plastic trays (diameter 30 cm and height 10 cm) were filled with inert quartz particle of size 0.4–0.6 mm soaked in 0.5 N HCl for 24 h prior use and washed thoroughly with deionized water until the pH of the sand became neutral. After filling the tray with oven dried quartz sand of 5 kg total weight (tray + sand) was recorded. Sterilized seeds were germinated after 0.5 % Carbendazim (Bavistin^R) treatment under environmental controlled chamber (Gyromax™737, Amerex instruments Inc, California), which were later transferred to quartz sand filled plastic trays. The volume of the nutrient solution was maintained as suggested by Baruah and Borthakur [28]. The concentrations of essential nutrients viz. N, K, Ca, P, S, Mg, Cl, B, Mn, Zn, Cu, Mo and Fe present in Hoagland solution (full strength) were 224, 235, 160, 62, 32, 24, 1.77, 0.27, 0.11, 0.13, 0.03, 0.05 and 1.00–2.00 mg/l, respectively. The pH of the working solution was maintained at 4.5. At initial stage of crop growth (up to 10 days after sowing), maize seedlings were nourished with Hoagland solution of half strength by manual pouring using beaker at an interval of two days and thereafter seedlings were fed with full strength Hoagland solution up to 40 days on 3 days interval. Hoagland solution with different levels of Al was prepared by adding desired quantity of aluminum chloride salt ($AlCl_3$, Merck). Seven concentrations of Al in the modified Hoagland solutions were T0: 0 μM Al, T1: 50 μM Al, T2: 100 μM Al, T3: 150 μM Al, T4: 200 μM Al, T5: 250 μM Al, T6: 300 μM Al were maintained with three replicates (Supplementary Fig. 1A).

Thirty one including local check maize cultivars were screened at selected three Al levels 0, 150 and 250 μM with three replicates, using plastic trays containing quartz sand were used similar to above experiment (Supplementary Fig. 1). The amount of solution (with Al) applied to each of the trays was assessed and applied at an interval of 3 days based on weight loss from the plastic tray which was compensated by adding Hoagland solution without having Al.

2.2.2. Soil culture experiment

Plastic pots were filled with air dried surface soil (0–15 cm) which was finely grounded, sieved (2 mm) and mixed thoroughly at fifteen days prior to sowing. The soil collected was sandy clay loam. Soil properties viz., organic carbon (%), available N, P, K (kg/ha), readily soluble aluminum (ppm) and exchangeable acidity (meq/100g soil) were 0.98. 400.4, 11.68, 121.8, 304.3 and 2.25, respectively. Soil moisture was maintained at 43.5 %. Ten cultivars along with check variety (RCM-1-76) were replicated for five times by following completely randomized design (CRD). Three plants of maize was maintained in each pot and physio-morphological stress indicators were assessed after 45 days of trail.

2.3. Physio-morphological traits

Representative plant samples were collected from each replicated pot having three plants after 45 days of sowing (DAS) from each tray for assessing physio-morphological traits following standard protocols. Chlorophyll and carotenoid pigment levels in fresh and matured leaves were estimated by acetone extraction method as described by Misyura et al. [29] (Supplementary material) whereas anthocyanin was estimated following the protocol described by Das et al. [30].

2.3.1. Measurement of photosynthetic and transpiration rate

Photosynthetic and transpiration rate measured according to Hazarika et al. [31]. Photosynthetic rate (A), and transpiration rate (E) were recorded by using portable photosynthesis system (IRGA- GFS Walz-6300, Germany). The measurements were done in the fully expanded leaf of randomly selected representative plants. Photosynthetic and transpiration rates are expressed in μmol CO_2/m^2/s and μmol H_2O/m^2/s.

2.3.2. Estimation of tissue phosphorus and Al

Tissue P concentration was determined by using di-acid mixture digestion followed by Vanadomolybdate phosphoric yellow method [32]. The intensity of the stable yellow colour of the liquid was determined using a spectrophotometer (Spectra scan UV-2600, Thermo Scientific, USA) at 450 nm wavelength and concentration of P was read from the standard curve. The concentration of Al in the filtrate was determined by using Atomic Absorption Spectroscopy (AAS) (Thermo fisher, ICE3500) at 309 nm wavelength after tissue digestion.

2.3.3. Determination of bio-concentration factor (BCF) and translocation factor (TF)

Bio-concentration factor (BCF) being important attribute to determine ability of plants for elemental accumulation during metal toxicity studies, it was calculated in the current study by dividing Al concentration in tissue (both root and shoot) with Al concentration in growing media. Translocation factor being another important tool used to assess plant potential for stress tolerance; it was calculated by metal concentration in shoot by root.

2.3.4. Rhizosphere acidification

Rhizosphere acidification of intact roots was assessed using modified protocol described by Romheld et al. [33] and Das et al. [30].

The uprooted maize plants were thoroughly washed with de-ionized water (DIW) followed by air drying for 15–20 min. Agar media (pH 6) containing 0.75 % (w/v) agar, 0.008 % (w/v) bromocresol purple, 2.5 mM K_2SO_4 and 1 mM $CaSO_4$ was prepared and solidified in autoclaved petri-plate upon which intact maize roots were gently spread and pressed with minimum root damage. These petri-plates were then transferred to growth chamber under sufficient light (~600 μE/m^2/S) and humidity (~70 %) for 24 h. The visual change in color of the agar medium surrounding the roots was qualitatively recorded and presented (Supplementary Fig. 2A). Hematoxylin staining of root of 25 days old maize seedlings was carried out as mentioned by Polle et al. [34]. The hematoxylin stain was prepared by dissolving 0.02 g hematoxylin stain and 0.02 g of KIO_3 in 100 ml of water and the solution was kept overnight. Uprooted maize plants were washed with de-ionized water and dried on tissue paper which was later immersed in hematoxylin stain for 30 min. After staining roots were rinsed with deionized water and dried. The traverse root section of roots tips (2 mm from distal end) were observed under compound microscope. The extent of color complex formed was captured under 40x compound microscope (Leica DM 750) (Supplementary Fig. 2B). The intensity of the bluish brown color indicates the degree of entry of Al and possible damage to the root cells by Al toxicity.

2.4. Maize cultivar screening for Al toxicity evaluation

A total of 31 maize cultivars including local check were subjected to Al stress screening using previously standardized sand culture. These cultivars were evaluated based on key physio-morphological parameters (approx 22no.), including root dry weight (RDW), shoot dry weight (SDW), root-to-shoot ratio (R/S ratio), total dry matter (TDM), shoot length (SL), root surface area (RSA), root diameter (RD), total root length (TRL), root volume (RV), leaf area (LA), leaf perimeter (LPM), specific leaf weight (SLW), chlorophyll *a* (Chl a), chlorophyll *b* (Chl b), total chlorophyll, carotenoids, anthocyanin, chlorophyll *a*/b ratio, total carotenoids/total chlorophyll ratio, cell membrane stability (CMS), photosynthetic rate (A), and transpiration rate (E). These cultivars were then assessed for their tolerance to aluminum toxicity by sorting individual parameter data at selected levels of aluminum stress (0, 150, and 250 μM). Frequency distribution of genotypes is constructed based on number of classes (no. of classes = 2.5 x $n^{1/4}$; n = total no. of parameters) by following Yule's formula and class interval which was calculated using the equation (Eq. 1) given below:

$$C.I = \frac{(Maximum\ value\ in\ the\ given\ data^{*} - Minimum\ value\ in\ the\ given\ data^{*})}{Number\ of\ classes} \quad (1)$$

* Maximum and minimum value for a particular observation mentioned above.

After determining number of classes and class intervals, data for each parameter was categorized and assorted into six different classes. Then the mean value of each parameter was grouped and assigned score of that particular class interval where that individual value lies. Based on the total score, cultivars were classified into three categories viz. tolerant, moderately tolerant and sensitive to Al toxicity. Under each class, three cultivars were selected depending on the total score obtained by summing up of the individual score determined for each of the parameters by following standard statistical method [35].

2.5. Statistical analysis

The data were analyzed by one-way ANOVA using SPSS statistical software. The mean values of the parameters under different treatments were compared using the Tukey's-b test of DMRT at $p = 0.05$ [36]. Screening experimental data were analyzed through two-factor analysis using the OP STAT (CCS HAU, Hisar, Haryana, India) [37]. The differences between the treatment means were tested for their statistical significance using the least significant difference (LSD) at $p = 0.05$. The Principal component analysis of experimental data was performed to calculate varied eigen values and to summarize more information in terms of variance [38].

3. Results

3.1. Physio-morphological response of check variety towards Al stress

To identify critical, tolerable and toxic levels of Al, different physiological stress indicators (such as chlorophyll (chl) a, chl b, total chl, carotenoids, anthocyanin, shoot and root characteristics, etc.) for RCM-1-76 (local check) have been studied under graded levels of

Table 1
Leaf pigment contents of maize (RCM-1-76) plants grown at different concentration of Al at the end of stress period (mean±SER).

Treatment	Chlorophyll *a* (mg/g)	Chlorophyll *b* (mg/g)	Total chlorophyll (mg/g)	Carotenoids (μg/g)	Anthocyanin (μg/g)
Control	0.80 ± 0.00^{a}	0.23 ± 0.006^{a}	1.07 ± 0.01^{a}	47.76 ± 1.53^{ab}	80.37 ± 2.91^{d}
50 μM	0.56 ± 0.00^{c}	0.20 ± 0.002^{b}	0.80 ± 0.02^{b}	43.31 ± 0.86^{b}	130.25 ± 1.51^{b}
100 μM	0.43 ± 0.01^{d}	0.13 ± 0.003^{c}	0.60 ± 0.02^{c}	33.67 ± 0.33^{c}	158.97 ± 3.23^{a}
150 μM	0.65 ± 0.02^{b}	0.20 ± 0.003^{b}	0.83 ± 0.00^{b}	51.62 ± 2.06^{a}	149.81 ± 3.05^{a}
200 μM	0.37 ± 0.01^{d}	0.14 ± 0.005^{c}	0.51 ± 0.02^{d}	32.89 ± 1.25^{cd}	97.82 ± 2.46^{c}
250 μM	0.20 ± 0.01^{e}	0.13 ± 0.007^{c}	0.32 ± 0.01^{e}	27.90 ± 1.24^{de}	130.16 ± 2.74^{b}
300 μM	0.16 ± 0.00^{e}	0.09 ± 0.004^{d}	0.27 ± 0.01^{e}	26.40 ± 0.87^{e}	108.77 ± 3.99^{c}

Note. Means with in a column followed by common letter/s are not significantly different ($p < 0.05$) as determined by Turky's b Test.

Al under sand culture experiment. Among physiological traits leaf pigments like total chlorophyll (1.07 and 0.83 mg/g) and carotenoids (47.7 and 51.6 μg/g) was found higher under control and moderate Al stress (150 μM), respectively (Table 1). Leaf chl a and chl b have decreased with increasing concentration of Al from 0 to 100 μM and at 150 μM, the values increased which again declined significantly with increasing concentration of Al. Table 1 reveals that the carotenoid content for check variety treated with 150 μM Al was 95.5, 85.0, 56.9, 34.7 and 19.1 % higher than plants treated with 300, 250, 200, 100 and 50 μM of Al levels, respectively. Anthocyanin content was 80.37 μg/g in case of check variety with no Al toxicity while it reached 159 μg/g in case of plants treated with 100 μM levels of Al (Table 1). However, there was no significant difference in anthocyanin content at 100 and 150 μM Al toxicity for the same variety. From Fig. 1A it was evident that chl a to b ratio has decreased with the advancement of Al stress till 50 μM and later increased with increase of Al stress till 150 μM while the ratios of accessory stress pigment to primary pigments has increased with increase of Al concentration (Fig. 1B).

Leaf parameters viz. leaf area, leaf dry weight, and leaf perimeter were significantly higher under control. From Table 2 it was observed that both leaf area and perimeter declined by 81.3 % and 62.4 % at 300 μM Al over control, respectively. Effect of graded dose of Al on specific leaf weight (SLW) was not significant up to 150 μM Al, however, it was significantly reduced thereafter (Table 2). Maximum shoot length of about 76.5 cm was observed under control and the lowest of 39.9 cm as recorded for 300 μM Al implying reduction of 6.8–47.8 % (Table 2). As evident from Table 3 that cell membrane stability (CMS) was higher (85.2 %) for control while the maximum cell membrane leakage (94.2 %) was observed for 300 μM Al stress with a progressive decreased in CMS with increasing in Al toxicity. The photosynthetic rate has decreased with increasing Al stress and highest being found under control (21.7 μmol/m^2/s) and lowest value (8.1 μmol/m^2/s) under 300 μM of Al reflecting gradual reduction of 13.5–62.6 %. However, there was a significant increase in transpiration rate initially with increasing dose of Al from 0 to 100 μM which significantly declined later with further increase in Al stress. It was highest (1.6 μmol/m^2/s) for 100 μM Al stress and lowest (0.73 μmol/m^2/s) in case of 300 μM Al stress (Table 3).

Total root length, root surface area, root diameter and root volume for check variety was maximum under without Al toxicity which significantly decreased at 300 μM Al (Table 4) to the tune of 92.9 %, 98.8 %, 92.6 % and 97.5 % over control, respectively. Although root dry weight was highest at 50 μM however it was not statistically significant compared to 100 and 150 μM Al (Table 4). The change in the colour intensity of basic media indicates rhizosphere acidification by maize seedling which was higher in 150, and 200 μM of Al (Fig. 4A and Supplementary Fig. 2A). Minimal colour change was observed in the agar media with 0 μM Al (Fig. 4A). However, treatment 250 and 300 μM indicated no marked change in colour of the agar media. Hematoxylin staining of roots reveals the extent of Al accumulation at intracellular space in root cortex was higher upon Al toxicity stress (Fig. 4B). The intensity of dark blue colour increased with the increase in Al concentration which depicts higher Al entry and compartmentation to root cell apoplast. The colour intensity was highest at 300 μM compared to control due to increase in Al absorption and deposition in root cells (Fig. 4B and Supplementary Fig. 2B). However, root to shoot (R/S) ratio was noticed significantly higher under 50 μM Al stress which was not significantly different with plants grown under 100, 150 and 300 μM. Lower R/S ratio (0.32) was recorded in 200 μM Al stress level.

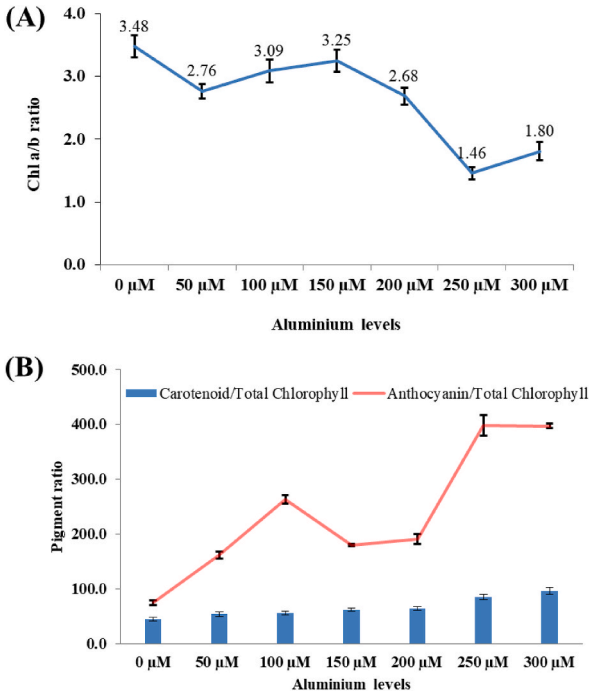

Fig. 1. Effect of aluminum stress levels on chlorophyll *a*/b ratio(A) and carotenoid to total chlorophyll, anthocyanin to total chlorophyll (B) at the end of Al stress exposure in maize (RCM -1-76) under sand culture assay.

Table 2
Effect of graded dose of Al on the shoot characteristics of maize (RCM-1-76) harvested at the end of stress period under sand culture (mean±SER).

Treatment	Leaf area (cm^2)	Leaf perimeter (cm)	Specific leaf weight (mg/cm^2)	Shoot dry weight (g)	Shoot length (cm)	Root to shoot ratio
Control	210.72 ± 6.70^{a}	121.89 ± 3.31^{a}	2.91 ± 0.23^{a}	2.23 ± 0.36a	76.50 ± 1.89^{a}	0.51 ± 0.12^{ab}
50 μM	180.13 ± 3.77^{b}	109.82 ± 1.64^{b}	2.80 ± 0.19^{a}	1.86 ± 0.10 ab	71.3 ± 4.81^{ab}	0.77 ± 0.05^{a}
100 μM	150.59 ± 0.34^{c}	101.25 ± 1.83^{c}	2.76 ± 0.11^{a}	1.87 ± 0.09 ab	64.16 ± 8.09^{abc}	0.58 ± 0.04^{ab}
150 μM	136.94 ± 3.35^{c}	92.70 ± 1.45^{d}	3.02 ± 0.19^{a}	1.82 ± 0.30 ab	60.23 ± 9.66^{abc}	0.54 ± 0.04^{ab}
200 μM	96.07 ± 2.84^{d}	76.43 ± 1.79^{e}	1.57 ± 0.11^{c}	1.31 ± 0.06BCE	56.63 ± 1.63^{abc}	0.32 ± 0.01^{b}
250 μM	73.23 ± 2.09^{e}	64.20 ± 1.33^{f}	1.68 ± 0.13^{bc}	0.70 ± 0.08cd	47.86 ± 1.59^{bc}	0.40 ± 0.05^{b}
300 μM	39.35 ± 2.06^{f}	41.27 ± 0.63^{g}	2.29 ± 0.13^{ab}	0.47 ± 0.02d	39.9 ± 2.05^{c}	0.50 ± 0.03^{ab}

Note. Means with in a column followed by common letter/s are not significantly different ($p < 0.05$) as determined by Turky's b Test.

Table 3
Effect of graded dose of Aluminium on the agro-physiological parameters at active growth stage of maize (RCM-1-76) (mean±SER).

Treatment	Photosynthetic rate, A ($\mu mol/m^2/s$)	Transpiration rate, E ($\mu mol/m^2/s$)	CMS (% leakage)	Total Dry Matter (g)
Control	21.75 ± 0.64^{a}	1.28 ± 0.03^{cd}	85.18 ± 1.89^{c}	3.32 ± 0.38^{a}
50 μM	18.82 ± 0.19^{b}	1.42 ± 0.01^{bc}	89.19 ± 0.74^{b}	3.31 ± 0.27^{a}
100 μM	17.35 ± 0.40^{c}	1.62 ± 0.02^{a}	91.04 ± 0.57^{ab}	2.95 ± 0.09^{a}
150 μM	13.69 ± 0.41^{d}	1.44 ± 0.03^{b}	90.41 ± 0.45^{ab}	2.79 ± 0.42^{a}
200 μM	11.70 ± 0.34^{e}	1.14 ± 0.05^{d}	92.93 ± 0.34^{ab}	1.75 ± 0.10^{b}
250 μM	10.26 ± 0.15^{e}	0.90 ± 0.03^{e}	93.91 ± 0.37^{a}	0.98 ± 0.08^{b}
300 μM	8.14 ± 0.21^{f}	0.73 ± 0.04^{f}	94.16 ± 1.01^{a}	0.71 ± 0.02^{b}

Note. Means with in a column followed by common letter/s are not significantly different ($p < 0.05$) as determined by Turky's b Test.

Table 4
Effect of graded dose of Al on the root characteristics at active growth stage of maize (RCM-1-76) (mean±SER).

Treatment	Total root length (cm)	Surface area (cm^2)	Root volume (cm^3)	Root diameter (mm)	Root dry weight (g)
Control	194.74 ± 16.26^{a}	255.84 ± 22.97^{a}	26.02 ± 1.98^{a}	3.31 ± 0.35a	1.08 ± 0.21^{a}
50 μM	144.71 ± 7.13^{b}	173.27 ± 4.15^{b}	21.54 ± 0.85^{b}	2.20 ± 0.32b	1.45 ± 0.17^{a}
100 μM	127.59 ± 6.00^{b}	155.18 ± 0.65^{b}	12.42 ± 0.78^{d}	2.09 ± 0.23b	1.08 ± 0.56^{a}
150 μM	83.52 ± 5.16^{c}	64.91 ± 9.43^{c}	17.05 ± 0.58^{c}	1.97 ± 0.26b	0.97 ± 0.13^{a}
200 μM	32.790 ± 1.58^{d}	21.56 ± 1.64^{d}	14.29 ± 0.53^{cd}	1.03 ± 0.07c	0.43 ± 0.42^{b}
250 μM	19.32 ± 1.75^{d}	4.47 ± 0.48^{d}	8.04 ± 0.30^{e}	0.96 ± 0.09c	0.27 ± 0.003^{b}
300 μM	14.33 ± 1.19^{d}	3.12 ± 0.08^{d}	0.65 ± 0.17^{f}	0.65 ± 0.02c	0.23 ± 0.003^{b}

Note. Means with in a column followed by common letter/s are not significantly different ($p < 0.05$) as determined by Turky's b Test.

Significantly increased root Al was observed with >200 μM Al (Table 5) and there was a two-fold increase in the Al at 300 μM compared to 50 μM Al. Maximum shoot Al (0.22 mg/gm) was observed at 300 μM Al. Increase in the ratio of shoot Al to root Al was observed with increase of Al stress up to 100 μM Al which later declined as shown in Fig. 2A. Root and leaf P were maximum (0.83 % and 0.89 %) at 200 μM Al stress and minimum under control treatment (0.15 % and 0.09 %) (Table 5), respectively. P levels at 250 and 300 μM Al in the root was moderate but shoot accumulation has reduced significantly. The bio-concentration factor (BCF) for Al

Table 5
Effect of graded levels of Al dose on the Al & P accumulation at active growth stage of maize (RCM-1-76) (mean±SER).

Treatment	Root Al (mg/gm)	Shoot Al (mg/gm)	Root P (%)	Shoot P (%)	BCF (AL)	BCF (P)	TF (Al)	TF (P)
Control	0.00 ± 0.000^{d}	0.00 ± 0.000^{d}	0.15 ± 0.00^{d}	0.09 ± 0.00^{e}	0.00 ± 0.000^{d}	0.00 ± 0.000^{d}	0.50 ± 0.500^{ab}	0.63 ± 0.074^{bc}
50 μM	0.19 ± 0.071^{c}	0.13 ± 0.014^{c}	0.44 ± 0.04^{c}	0.46 ± 0.05^{cd}	0.26 ± 0.016^{a}	0.01 ± 0.001^{c}	0.62 ± 0.128^{ab}	1.09 ± 0.365^{ab}
100 μM	0.21 ± 0.002^{c}	0.19 ± 0.012^{ab}	0.45 ± 0.01^{c}	0.50 ± 0.01^{c}	0.14 ± 0.019^{b}	0.02 ± 0.001^{c}	1.01 ± 0.051^{a}	1.12 ± 0.044^{ab}
150 μM	0.28 ± 0.014^{c}	0.16 ± 0.007^{bc}	0.58 ± 0.03^{bc}	0.45 ± 0.03^{cd}	0.11 ± 0.009^{c}	0.02 ± 0.000^{c}	0.57 ± 0.005^{ab}	0.79 ± 0.206^{bc}
200 μM	0.30 ± 0.043^{c}	0.21 ± 0.004^{a}	0.83 ± 0.06^{a}	0.89 ± 0.03^{a}	0.10 ± 0.013^{c}	0.03 ± 0.001^{a}	0.73 ± 0.190^{ab}	1.09 ± 0.224^{ab}
250 μM	0.44 ± 0.010^{b}	0.15 ± 0.002^{c}	0.55 ± 0.03^{bc}	0.74 ± 0.03^{b}	0.09 ± 0.003^{c}	0.02 ± 0.000^{b}	0.34 ± 0.011^{b}	1.37 ± 0.236^{a}
300 μM	0.56 ± 0.057^{a}	0.22 ± 0.009^{a}	0.62 ± 0.02^{b}	0.34 ± 0.04^{d}	0.10 ± 0.011^{c}	0.02 ± 0.002^{c}	0.40 ± 0.100^{b}	0.54 ± 0.095^{c}

Note. Means with in a column followed by common letter/s are not significantly different ($p < 0.05$) as determined by Turky's b Test. Bio-concentrations factor, TF – translocation factor.

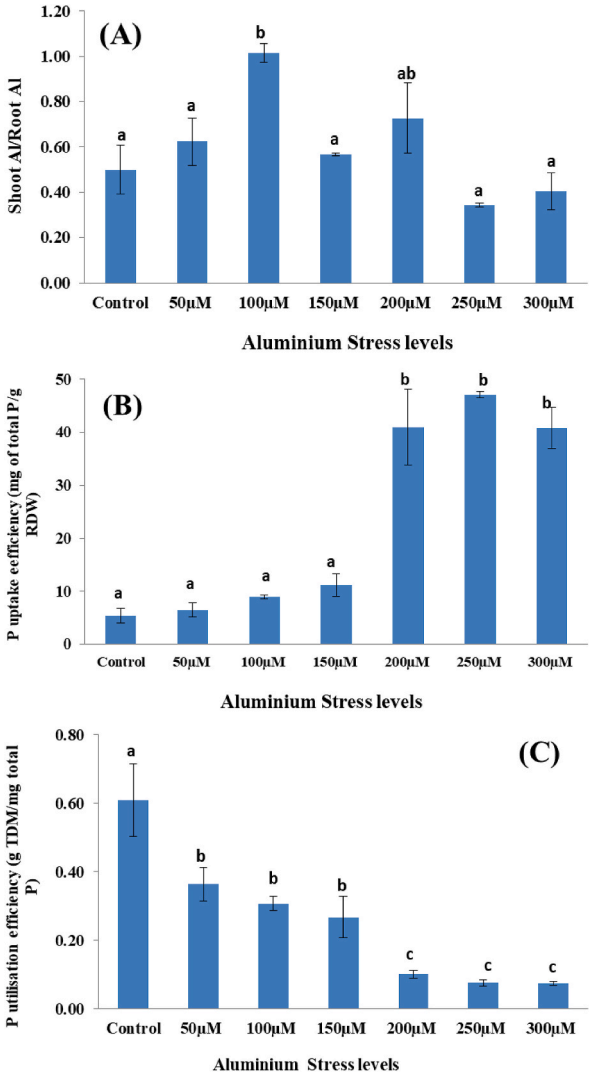

Fig. 2. Effect of graded levels of aluminum stress on shoot Al to root Al ratio (A), P Uptake and P utilization efficiency (B and C) of maize local check under sand culture.

toxicity has been differed significantly between the control and treatments with 50 μM and 100 μM of Al. However, there was no significant difference observed among treatments with 150 μM, 200 μM, 250 μM, and 300 μM of Al. This suggests that the uptake and accumulation of Al in the roots varied notably at lower concentrations of Al compared to the control, but beyond 100 μM (Table 5). BCF for P was differed significantly between the control group and 50 μM, 100, 250 and 300 μM of Al. Maximum P uptake efficiency was recorded for 250 μM of Al stress (Fig. 2B), however, the P utilization efficiency (PUE) was substantially reduced with increase in concentration of Al application, maximum being observed under control and the lowest was observed at 300 μM Al (Fig. 2C). Interestingly, the PUE of plants grown under control was almost ~9 times higher than that under 300 μM Al stress. Leaf perimeter (LP) showed strong positive correlation with root and shoot morphological traits (root dry weight (RDW), root length (RL), shoot length (SL) and shoot dry weight (SDW) as evident from Fig. 3A.

3.2. *Principal component analysis (PCA) for genotype screening*

The Principal component analysis of experimental data revealed a total five principal components are having eigen values > 1 (Tables 6A and 6B). These components were retained since they were having eigen values greater than the average eigen value (0.999) as they summarize more information in terms of variance [38]. From Fig. 3B it is evident that the curve attained an elbow shape or flattened at PC5 suggesting that >80 % variance was explained by first 5 PCs. From the table of PCA loadings (Table 6A) the parameters SDW, TDM, RL, Total root length (TRL), SL and Leaf area (LA) contributed 46.02 % of variability to PC1, whereas photosynthetic rate (A), root volume (RV), root surface area (RSA), anthocyanin and Chl b, contributed 13.56 % of variability to PC2. In case of PC3,

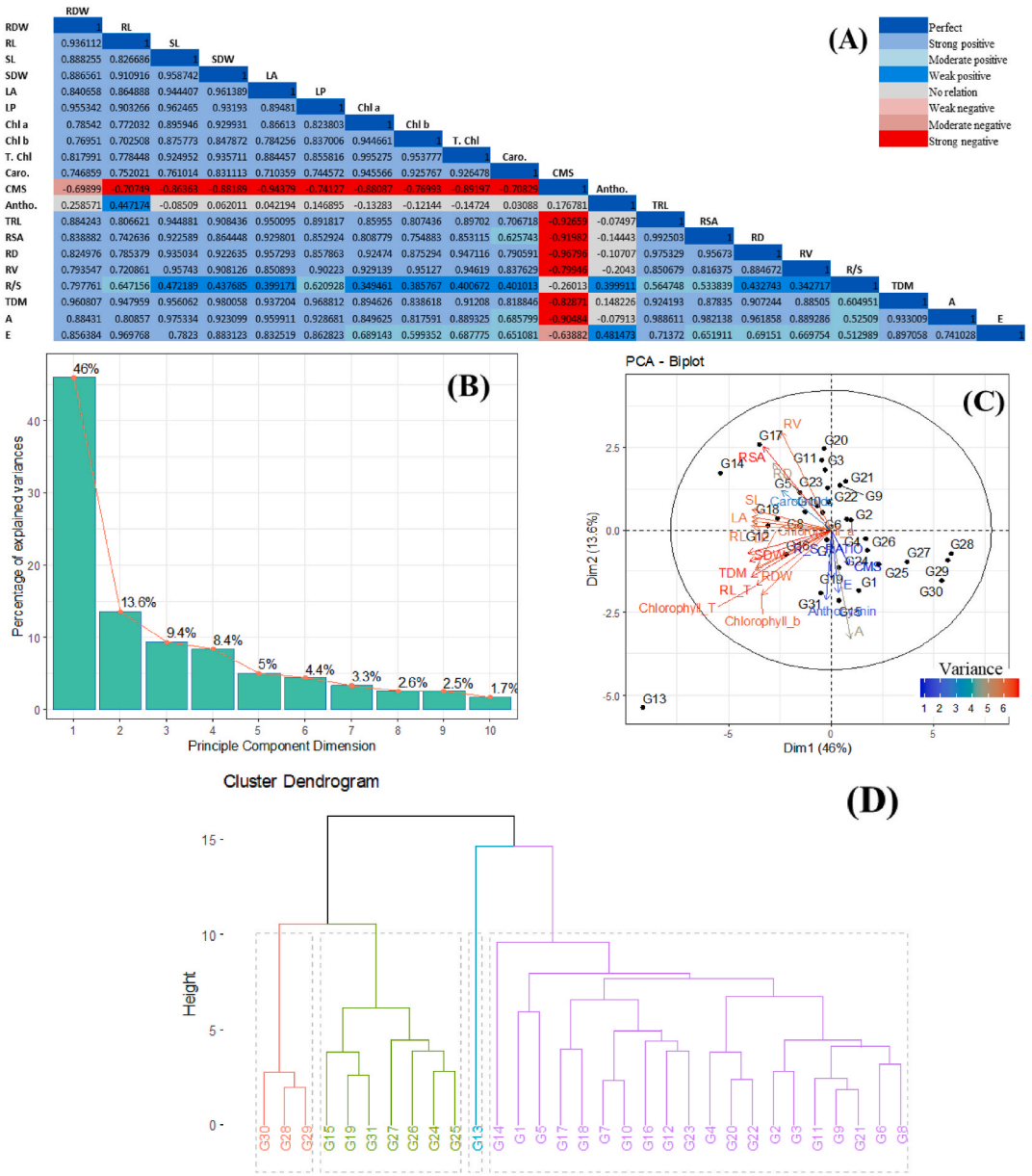

Fig. 3. Heat map showing Pearson's correlation coefficients for biomass traits (A), Screeplot of different physiological parameters for twenty genotypes (B), PCA biplot of different physiological parameters for thirty one genotypes (C),and the dendrogram cluster of thirty one genotypes based onphysiological parameters (D).

Transpiration rate (E), root diameter (RD), RDW, A, TDM and anthocyanin contributed 9.41 % of total variability, whereas CMS, R/S ratio, E and anthocyanin contributed 8.44 % of variability to PC 4. In case of PC5, E, RV, Chl a, Chl b, Total Chl, and anthocyanin contributed 5.05 % of total variability. The PCA outcome indicated strongly A, RV, RSA, anthocyanin and Chl b, Total Chl, and TDM were emanated as key stress physiological traits for evaluating thirty one genotypes with higher of divergence values while the other remaining characters were comparatively less contributing factors for divergence; thus, these traits should be kept in consideration during the selection of Al stress tolerating cultivars in Maize.

From the PCA biplot diagram (Fig. 3C), two PCA components had been depicted as PC1 = Dimension 1 (Dim 1) and PC 2 = Dimension 2 (Dim 2). The data set represented a focused total of twenty arrows to depict each variable. The variable CMS, R/S ratio, A, E shows positive loading, whereas RDW, RL, SL, SDW, LA, LP, Chl a, Chl b, Total Chl, carotenoids, anthocyanin, TRL, RSA, RD, RV and TDM exhibit negative loadings for PC1. Similarly, for PC2 the variable RDW, SDW, Chl a, Chl b, Total Chl, CMS, anthocyanin, TRL, R/S ratio, TDM, A, E exhibit negative loadings except for RL, SL, LA, LP, carotenoids, RSA, RD, and RV. SDW (0.293), TDM (0.288), RL (0.281), SL (0.279) are close to each other, indicating a higher positive correlation between them.

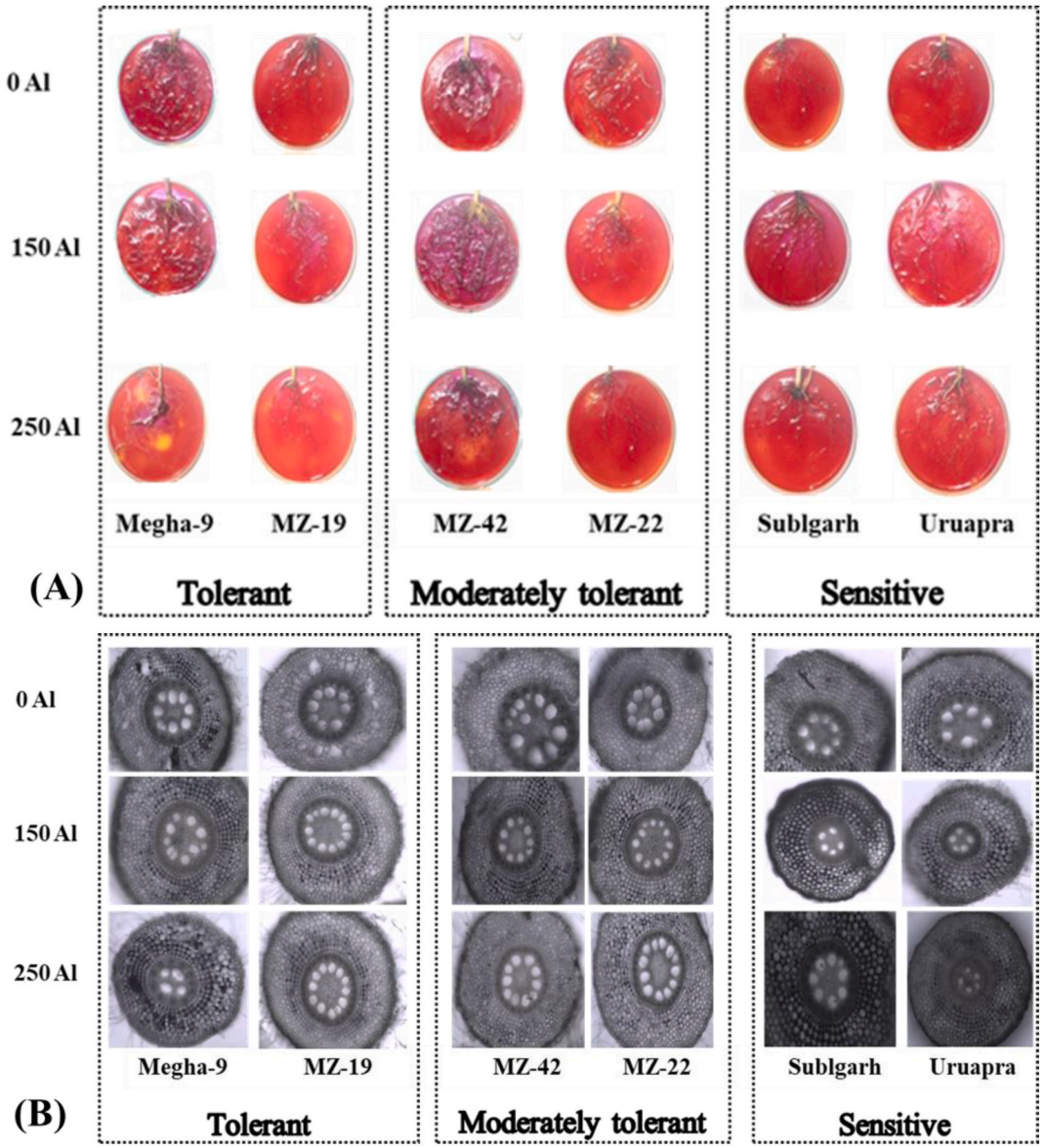

Fig. 4. Effect of selected levels of aluminum stress on root acidification (A) and Al accumulation after hematoxylin staining (B) in identified lines of maize under sand culture.

From the PCA biplot diagram (Fig. 3C), two PCA components are represented as PC1 = Dimension 1 (Dim 1) and PC2 = Dimension 2 (Dim 2). The dataset is depicted with a total of twenty arrows representing each variable. Variables such as CMS, R/S ratio, A, and E exhibit positive loadings, while RDW, RL, SL, SDW, LA, LP, Chl a, Chl b, Total Chl, carotenoids, anthocyanin, TRL, RSA, RD, RV, and TDM show negative loadings for PC1. Similarly, for PC2, variables including RDW, SDW, Chl a, Chl b, Total Chl, CMS, anthocyanin, TRL, R/S ratio, TDM, A, and E exhibit negative loadings, except for RL, SL, LA, LP, carotenoids, RSA, RD, and RV. SDW (0.293), TDM (0.288), RL (0.281), and SL (0.279) are closely correlated, indicating a higher positive correlation among them.

Clustering was performed for a total of thirty one genotypes, revealing four main clusters (Fig. 3D). In the first cluster, only sensitive cultivars viz. Uruapara, Sublgarh, and BRL Para (G30, G28, and G29 respectively) are placed. The second cluster includes genotypes viz., moderately tolerant cultivar (Megh 11, MZM-42, Local check, SKMP, LNG Local, KNS Local and TRE local having genotype number like G15, G19, G31, G27, G26, G24, and G25 respectively. The third cluster solely consists of highly Al stress tolerant cultivar viz., Megh 11 with genotype no. G13, while the fourth cluster comprises twenty different genotypes with varied Al toxicity stress tolerance: G14, G1, G5, G17, G18, G7, G10, G16, G12, G23, G4, G20, G22, G2, G3, G11, G9, G21, G6, and G8, respectively. The highest mean values for CMS, SL, LP, LA, RL, and anthocyanin are recorded in cluster I, while the highest mean values for CMS, SL, LA, LP, RLT, and RSA are found in cluster II. Cluster III records the highest mean values for RL_T, RSA, LP, LA, CMS, and SL, whereas cluster IV shows the highest values for RSA, RL_T, CMS, LP, LA, and SL, respectively. The correlation heat maps have been provided in the

Table 6
A: PCA loadings of different parameters used for screening of maize genotypes
B: Eigen value, variance% and cumulative variance of different PCA loadings of Maize screening experiment.

Parameters	PC1	PC2	PC3	PC4	PC5
RDW	−0.271	−0.164	−0.288	−0.001	0.152
RL	−0.281	0.015	0.162	−0.127	0.051
SL	−0.279	0.083	−0.148	−0.103	0.137
SDW	−0.293	−0.093	−0.168	0.122	0.165
LA	−0.278	0.053	−0.026	−0.209	0.142
LP	−0.277	0.037	−0.053	−0.26	0.054
Chl a	−0.264	−0.154	0.23	0.018	−0.259
Chl b	−0.246	−0.262	0.14	0.127	−0.404
ChlT	−0.264	−0.222	0.188	0.085	−0.359
Carotinoids	−0.174	0.159	−0.24	0.144	−0.262
CMS	0.062	−0.14	0.127	0.577	0.199
Anthocyanin	−0.016	−0.281	−0.224	0.366	0.321
TRL	−0.281	−0.19	0.046	0.094	0.023
RSA	−0.238	0.337	0.114	0.079	0.207
RD	−0.207	0.271	0.394	0.033	−0.018
RV	−0.175	0.394	0.141	0.118	0.253
R/S ratio	0.001	−0.195	−0.31	−0.453	−0.031
TDM	−0.288	−0.127	−0.227	0.067	0.162
A	0.067	−0.437	0.252	−0.088	0.211
E	0.025	−0.252	0.452	−0.298	0.394

Components	**Eigen value**	**variance %**	**cumulative variance%**
PCA 1	9.205	46.026	46.026
PCA 2	2.713	13.563	59.589
PCA 3	1.881	9.405	68.994
PCA 4	1.687	8.437	77.431
PCA 5	1.009	5.045	82.476

supplementary material.

3.3. Al stress tolerating potential of maize cultivars

Maize cultivars were screened for Al toxicity for three different Al concentration viz., 0, 150 and 250 μM Al by monitoring stress sensitive physio-morphological stress indicators at active crop growth period. Among all the cultivars Megha-9 has shown the highest total chl and chl b content comparatively (Supplementary Table 2A) across all the treatments whereas minimum value was recorded by SKM PM-2 under 150 μM Al; and *Sublgarh* and *Uruapara* cultivars (0.06 mg/g per plant) at 250 μM Al. In general, carotenoid level tends to decrease with increase in Al stress however; Megha-10, NEH-5 and LNG Local recorded the highest carotenoid content. Anthocyanin content was highest in case of Haz, NEH-5 and RCM-1-76 under control, 150 and 250 μM Al, respectively (Supplementary Table 2B). Higher leaf area was recorded by Megha-9, MZM-22 and Megha-10 under control, 150 μM and 250 μM respectively (Supplementary Table 3A), whereas higher leaf perimeter was recorded by cultivars like Megha-9 under 150 and 250 μM. NEH-5, Megha-9 and Megha-10 had maximum shoot length whereas BRL para was recorded lowest shoot length under all treatments (Supplementary Table 3B). Additionally, NEH-5, NEH-9 and LNG Local have shown higher CMS whereas MZM-17, Megha-9 and Megha-10 have recorded the lowest CMS under control, 150 and 250 μM conditions, respectively (Supplementary Table 4). Maize cultivars viz., NEH-5, Megha-8 and MZM-22 had recorded higher photosynthetic rate compared to other cultivars whereas SKM PM-2, *Sublgarh* and KNS Local have recorded lowest under control, 150 μM and 250 μM, respectively (Supplementary Table 5). Higher transpiration rate was observed in Megha-2, MZM-19 and NEH-5 whereas MZM-71, Megha-9 and Megha-11 recorded low transpiration rate in control, 150 μM and 250 μM, respectively.

Based on the scoring the extent of stress tolerance and stress damage at selected Al stress levels, screened maize cultivars were grouped into three categories viz. tolerant (with highest total score including Megh −9, Megh10 and MZM-19), moderately tolerant (including Megh-1, MZM-22 and MZM-42), and sensitive cultivars (with lowest score e.g., BRL para, *Subhalgarh* and *Uruapara*) (Supplementary Table 6). To further investigate specific trends, patterns, and relationships within that subset statistical analysis was performed again with selected genotypes. Tolerant cultivars have higher mean chl *a* and *b* content (121 % and 77.5 %) compared to sensitive cultivars (BRL para, *Subhalgarh* and *Uruapara*), respectively (Table 7). Level of carotenoids and anthocyanin content were higher in tolerant cultivars over sensitive (BRL para, *Subhalgarh* and *Uruapara*) by 62.7 % and 18.4 %, respectively (Table 7). However, the extent of decrease in carotenoid to total chl in tolerant, moderately tolerant and sensitive cultivars with increase in concentration of Al was 75.8 %, 31.1 % and 34.2 %, respectively (Table 7). CMS of tolerant, sensitive cultivars and check variety have decreased to the tune of 1.1 %, 1.9 % and 3.7 %, respectively with increase in Al stress from 0 to 250 μM (Table 8). The ratio of chl a/b, carotenoids to total chl and anthocyanin to total chl have reduced in the tolerant cultivars by 47.2 %, 10.9 % and 64.4 % over sensitive cultivars, respectively. Extent of reduction in photosynthetic and transpiration rate of the cultivars under 150 μM Al stress was to the tune of 8.0 and 45.3 % respectively compared to control (Table 8). Photosynthetic rate of tolerant, moderately tolerant and sensitive cultivars was

Table 7
Leaf pigment contents of contrasting maize cultivars grown for 40 days at different concentration of aluminium under sand culture.

Category	Cultivar	Chlorophyll *a* (mg/g)				Chlorophyll *b* (mg/g)				Carotenoids (μg/g)				Anthocyanin (μg/g)			
	Al (μM)	0	150	250	Mean	0	150	250	Mean	0	150	250	Mean	0	150	250	Mean
Tolerant	Megha-9	0.642	0.592	0.811	**0.68^{a}**	0.612	0.489	1.271	**0.79^{a}**	25.623	22.431	10.424	**19.49a**	15.915	23.911	25.217	**21.7^{a}**
	Megha-10	0.356	0.529	0.321	**0.40^{ab}**	0.229	0.255	0.163	**0.22^{b}**	82.584	26.616	9.048	**39.42a**	19.078	25.688	25.250	**23.3^{a}**
	MZM-19	0.259	0.434	0.662	**0.45^{b}**	0.155	0.168	0.244	**0.19^{b}**	14.188	24.958	20.985	**20.04a**	23.893	22.036	24.956	**23.6^{a}**
	Mean	**0.419**	**0.518**	**0.598**	**0.51**	**0.332**	**0.304**	**0.559**	**0.40**	**40.798**	**24.668**	**13.486**	**26.32**	**19.628**	**23.878**	**25.141**	**22.9**
Moderately Tolerant	Megha-1	0.268	0.283	0.428	**0.33^{b}**	0.217	0.171	0.180	**0.19^{b}**	22.453	12.496	12.986	**15.98a**	13.495	18.347	13.462	**15.1^{a}**
	Mizo-22	0.287	0.450	0.609	**0.45^{b}**	0.155	0.197	0.221	**0.19^{b}**	16.652	21.908	21.354	**19.97a**	18.164	12.145	25.963	**18.8^{a}**
	Mizo-42	0.082	0.338	0.418	**0.28^{b}**	0.100	0.184	0.160	**0.15^{b}**	3.701	17.734	15.060	**12.17a**	20.431	13.332	29.267	**21.0^{a}**
	Mean	**0.212**	**0.357**	**0.485**	**0.35**	**0.157**	**0.184**	**0.187**	**0.18**	**14.268**	**17.379**	**16.467**	**16.04**	**17.363**	**14.608**	**22.897**	**18.3**
Sensitive	BRL Local	0.066	0.393	0.208	**0.22^{b}**	0.060	0.135	0.075	**0.09^{b}**	7.816	18.738	12.095	**12.88a**	26.495	5.253	18.416	**16.7^{a}**
	Sublgarh	0.187	0.243	0.241	**0.22^{b}**	0.073	0.123	0.062	**0.09^{b}**	14.429	14.860	10.783	**13.36a**	20.823	10.562	21.629	**17.7^{a}**
	Uruapara	0.156	0.354	0.180	**0.23^{b}**	0.081	0.176	0.061	**0.11^{b}**	11.575	16.885	7.459	**11.97a**	21.447	19.432	24.072	**21.6^{a}**
	Mean	**0.136**	**0.330**	**0.210**	**0.23**	**0.071**	**0.145**	**0.066**	**0.09**	**11.273**	**16.827**	**10.113**	**12.74**	**22.922**	**11.749**	**21.373**	**18.7**
Check	RCM-76	0.347	0.618	0.367	**0.44^{b}**	0.064	0.221	0.165	**0.15^{b}**	3.288	15.307	10.826	**9.81a**	20.431	23.841	31.271	**25.2^{a}**
Factors		**C.D**	**SE(d)**	**SE(m)**	**F.V**	**C.D**	**SE(d)**	**SE(m)**	**F.V**	**C.D**	**SE(d)**	**SE(m)**	**F.V**	**C.D**	**SE(d)**	**SE(m)**	**F.V**
Factor (A)		0.053	0.027	0.019	**59*****	NS	1.175	0.831	1.7NS	2.377	1.185	0.838	103***	1.818	0.906	0.641	21***
Factor (B)		0.029	0.015	0.010	**81*****	NS	0.643	0.455	0.3NS	1.302	0.649	0.459	26.2***	0.996	0.497	0.351	56***
Factor (A*B)		0.092	0.046	0.032	**11*****	NS	2.035	1.439	1.2NS	4.117	2.053	1.452	95.3***	3.149	1.57	1.11	23***

Note. Factor A –Maize cultivar; B –Al stress level; F.V– F value; NS = not significant.

Table 8
Shoot characteristics of contrasting maize cultivars grown for 40 days at different concentration of aluminium under sand culture.

Category	Cultivar	Shoot length (cm)				Cell Membrane integrity (%)				Photosynthetic rate (μmol/m^2/s)				Transpiration rate (μmol/m^2/s)			
	Al (μM)	0	150	250	Mean	0	150	250	Mean	0	150	250	Mean	0	150	250	Mean
Tolerant	**Megha-9**	78.43	83.83	74.27	**78.84^{a}**	82.698	88.610	86.030	**85.78^{a}**	16.356	10.864	8.189	**11.80^{a}**	0.947	0.034	0.970	**0.65^{a}**
	Megha-10	79.60	75.03	88.00	**80.88^{a}**	82.946	82.783	78.752	**81.49^{a}**	10.283	9.076	8.490	**9.28^{a}**	0.806	0.346	0.592	**0.58^{a}**
	Mizo-19	67.60	73.13	74.37	**71.70^{ab}**	83.518	87.414	81.609	**84.18^{a}**	15.491	13.207	6.626	**11.77^{a}**	1.544	1.508	0.595	**1.22^{a}**
	Mean	**75.21**	**77.33**	**78.88**	**77.14**	**83.054**	**86.269**	**82.130**	**83.82**	**14.043**	**11.049**	**7.768**	**10.95**	**1.099**	**0.629**	**0.719**	**0.82**
Moderately Tolerant	**Megha-1**	78.90	65.70	68.90	**71.17^{abc}**	85.510	84.359	85.264	**85.04^{a}**	17.051	11.680	8.863	**12.53^{a}**	1.669	0.509	1.398	**1.19^{a}**
	Mizo-22	61.70	60.33	66.43	**62.82^{bc}**	82.290	82.332	87.358	**83.9^{a}**	11.117	10.648	3.708	**8.49^{a}**	0.402	0.379	0.332	**0.37^{a}**
	Mizo-42	57.50	62.80	66.53	**62.28^{bc}**	83.334	88.504	86.173	**86.00^{a}**	17.198	16.654	9.584	**14.48^{a}**	0.506	0.759	1.118	**0.79^{a}**
	Mean	**66.03**	**62.94**	**67.29**	**65.42**	**83.712**	**85.065**	**86.265**	**85.01**	**15.122**	**12.994**	**7.385**	**11.83**	**0.859**	**0.549**	**0.950**	**0.79**
Sensitive	**BRL Local**	30.17	22.00	25.50	**25.89^{e}**	85.921	85.219	85.890	**85.68^{a}**	6.799	6.478	3.927	**5.73^{a}**	1.667	0.907	0.794	**1.12^{a}**
	Sublgarh	36.37	40.10	39.70	**38.72^{d}**	87.511	82.484	85.247	**85.08^{a}**	6.441	5.351	9.329	**7.04^{a}**	1.150	1.010	0.613	**0.92^{a}**
	Uruapara	34.00	31.33	27.00	**30.78^{de}**	88.949	87.247	86.271	**87.49^{a}**	9.333	8.456	4.358	**7.38^{a}**	0.931	0.672	0.307	**0.64^{a}**
	Mean	**33.51**	**31.14**	**30.73**	**31.80**	**87.460**	**84.983**	**85.803**	**86.08**	**7.524**	**6.762**	**5.872**	**6.72**	**1.250**	**0.863**	**0.572**	**0.89**
Check	**RCM-76**	62.67	57.70	59.00	**59.79^{c}**	86.231	82.431	83.030	**83.90^{a}**	15.794	15.807	8.574	**13.39^{a}**	0.808	0.847	1.696	**1.12^{a}**
Factors		**C.D**	**SE(d)**	**SE(m)**	**F.V**	**C.D**	**SE(d)**	**SE(m)**	**F.V**	**C.D**	**SE(d)**	**SE(m)**	**F.V**	**C.D**	**SE(d)**	**SE(m)**	**F.V**
Factor (A)		7.35	3.67	2.59	**56*****	0.634	0.316	0.224	51***	0.548	0.237	0.193	224***	0.190	0.095	0.067	20.2***
Factor (B)		NS	2.01	1.42	**0.3NS**	0.347	0.173	0.123	10***	0.300	0.150	0.106	478***	0.104	0.052	0.037	3.6**
Factor (A*B)		NS	6.35	4.491	**0.8NS**	1.099	0.548	0.387	36***	0.949	0.473	0.335	54***	0.330	0.164	0.116	14.3***

Note. A –Maize cultivar; B –Al stress level, ; F.V– F value; NS = not significant.

Table 9
Root characteristics of contrasting maize cultivars grown for 40 days at different concentration of aluminium under sand culture.

Category	Cultivar	Total Root Length (cm)				Root Surface Area (cm^2)				Root Diameter (mm)				Root Volume (cm^3)			
	Al (μM)	0	150	250	Mean	0	150	250	Mean	0	150	250	Mean	0	150	250	Mean
Tolerant	**Megha-9**	315.85	179.95	1494.43	**663.41**	128.75	124.68	240.47	**164.64**	8.06	3.65	3.19	**4.97**	10.05	7.24	13.27	**10.19**
	Megha-10	284.41	66.64	255.48	**202.18**	709.97	29.89	166.75	**302.20**	6.01	2.54	3.00	**3.85**	134.29	1.19	7.62	**47.70**
	Mizo-19	297.38	78.11	212.31	**195.93**	837.01	76.32	127.23	**346.85**	9.33	4.90	2.69	**5.64**	226.70	5.93	5.58	**79.40**
	Mean	**299.21**	**108.24**	**654.07**	**353.84**	**558.58**	**76.96**	**178.15**	**271.23**	**7.80**	**3.70**	**2.96**	**4.82**	**123.68**	**4.79**	**8.82**	**45.76**
Moderately Tolerant	**Megha-1**	395.78	19.56	144.96	**186.77**	648.57	9.26	91.05	**249.63**	6.59	1.91	3.08	**3.86**	110.82	0.18	4.63	**38.54**
	Mizo-22	382.02	75.31	59.33	**172.22**	688.53	53.68	46.59	**262.93**	8.39	4.10	4.71	**5.73**	113.03	3.54	4.55	**40.37**
	Mizo-42	207.28	145.79	84.88	**145.98**	188.29	113.02	63.10	**121.47**	4.48	4.12	3.60	**4.06**	14.35	10.59	3.83	**9.59**
	Mean	**328.36**	**80.22**	**96.39**	**168.32**	**508.46**	**58.66**	**66.91**	**211.34**	**6.49**	**3.38**	**3.79**	**4.55**	**79.40**	**4.77**	**4.34**	**29.50**
Sensitive	**BRL Local**	9.13	19.93	11.97	**13.68**	12.57	2.77	2.91	**6.08**	1.91	1.11	1.23	**1.42**	0.23	0.03	0.05	**0.11**
	Sublgarh	8.77	17.53	13.73	**13.35**	17.90	2.44	4.35	**8.23**	1.92	1.42	1.34	**1.56**	0.20	0.03	0.07	**0.10**
	Uruapara	21.58	20.23	27.13	**22.98**	16.10	2.17	4.24	**7.50**	1.46	1.66	4.71	**2.61**	0.18	0.05	2.69	**0.97**
	Mean	**13.16**	**19.23**	**17.61**	**16.67**	**15.52**	**2.46**	**3.83**	**7.27**	**1.76**	**1.40**	**2.43**	**1.86**	**0.21**	**0.04**	**0.94**	**0.39**
Check	**RCM-76**	241.00	96.69	125.79	**154.49**	155.46	49.71	108.64	**104.60**	3.90	2.98	4.23	**3.70**	21.70	2.72	7.09	**10.50**
Factors		**C.D**	**SE(d)**	**SE(m)**	**F.V**	**C.D**	**SE(d)**	**SE(m)**	**F.V**	**C.D**	**SE(d)**	**SE(m)**	**F.V**	**C.D**	**SE(d)**	**SE(m)**	**F.V**
Factor (A)		339.37	169.23	119.66	**2.4****	22.67	11.30	7.99	241***	1.86	0.93	0.655	5.4***	4.29	2.14	1.51	298***
Factor (B)		NS	92.69	65.54	**1.5NS**	12.42	6.192	4.378	699***	1.02	0.507	0.359	6.2**	2.35	1.172	0.828	102***
Factor (A*B)		NS	293.12	207.27	**1.3NS**	39.26	19.58	13.846	201***	3.22	1.604	1.134	3.07***	7.43	3.705	2.62	318***

Note. A –Maize cultivar; B –Al stress level F.V– F value; NS = not significant. There was no statistical significance was observed among the cultivar groups and the parameters.

decreased to the extent of 45.1 %, 51.1 % and 21.9 %, respectively with the increase in concentration Al from 0 to 250 μM (Table 8).

Tolerant cultivars (Megha 9, Megha 10, and Mizo 19) have higher shoot length as compared to the sensitive cultivars (BRL para, *Subhalgarh* and *Uruapara*) and check variety to the tune of 58.7 % and 22.5 %, respectively whereas electrolyte leakage of tolerant cultivars was reduced up to 2.6 % over the sensitive cultivars as suggested by Table 8. Highest RSA was observed in Megha-9 under both 150 and 250 μM. Under 250 μM Al stress, cultivars Megha-10 and BRL Para have recorded the highest and lowest root volume (Supplementary Table 6A). The overall reduction in TRL at 150 and 250 μM Al was observed to be 70 % and 42.3 % in comparison to control (Table 9). From Table 9 and it was evident that tolerant cultivars have 20 and 2.3 fold increase in TRL and 36 and 2.6 fold increase RSA compared to sensitive cultivar and check variety, respectively. It was observed from Table 8 that tolerant maize cultivars have higher root diameter and root volume compared to sensitive cultivars and check variety by 62 % and 61.4 %; and 117 % and 4.4 fold, respectively. Majority of maize cultivars showed a higher intensity of media colour change at 150 μM Al compared to control and 250 μM Al stress. Based on the colour intensity of the media it can be assessed that maximum rhizosphere acidification occurred in Megha 9 under sand culture with 250 μM Al (Fig. 4A). Rhizosphere acidification of tolerant genotypes viz., Megh-9 and MZ-19 was indicatively maximum at 250 μM Al compared to rest of cultivars (Fig. 4A). The intensity of blue colour resulting from hematoxylin stain of root cell increased with increasing concentration of Al indicating higher accumulation of Al in the cell (Fig. 4B and Supplementary Fig. 2B). The extent of Al entry and accumulation was significantly higher in susceptible cultivars compared to other two types of cultivars (Fig. 4B).

Highest RDW was found in control and 250 μM for tolerant cultivars (Megha 9, Megha 10, and Mizo 19) whereas sensitive cultivars (BRL para, *Subhalgarh* and *Uruapara*) exhibited lowest RDW (Supplementary Table 7). Additionally, irrespective of the treatment, SDW was higher in case of tolerant cultivar whereas sensitive cultivars (*Uruapara and Sublgarh*) have shown least SDW. Similar results were observed in case of TDM, where tolerant maize cultivars recorded higher TDM compared to sensitive cultivars (Supplementary Table 7). The overall reduction in TDM of the cultivars for 150 and 250 μM Al stress was to the tune of 1.9 and 12.4 %, respectively compared to control. It was observed that tolerant cultivars had higher RDW, SDW and TDM over sensitive cultivars to the tune of 91 %, 87 %, and 88.7 %, respectively (Fig. 5A). RDW of tolerant and moderately tolerant cultivars increased to the extent of 49 % and 35.5 % with the increased of Al stress whereas in case of sensitive cultivars and check variety the decrease was 14.3 % and 57.9 % (Fig. 5A). TDM of tolerant and moderately tolerant cultivars has increased with increase in concentration of Al (77.8 % and 41.1 %, respectively) whereas sensitive cultivars and check variety a decline of 11.1 % and 46.9 % was noticed (Fig. 5A). Tolerant cultivars and moderately tolerant cultivars have higher R/S ratio over the sensitive cultivars (26.8 %) (Fig. 5B). Tolerant cultivars shown higher root Al compared to sensitive cultivars and check variety by 33.8 % and 40 %, respectively (Table 10). However, shoot Al content of tolerant cultivars was lower to the tune of 79.7 % and 143.2 % as compared to sensitive cultivars and check variety, respectively. Root Al content was in order of tolerant > moderately tolerant > sensitive cultivars and was amplified by 99, 189 and 68 % with increasing doses of Al to 300 μM. Higher P was observed in root and leaf tissue of tolerant cultivars i.e.,5.6 % and 23.8 % over sensitive and check variety (Table 10). The increase in root P content of tolerant and moderately tolerant cultivars was by 13.8 % and 5.9 % with increase

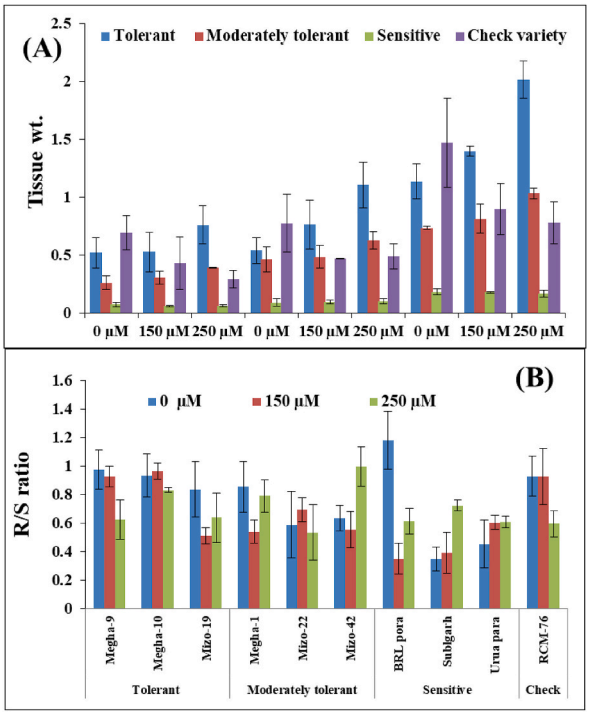

Fig. 5. Root and shoot dry weights (A) and root to shoot ratios (B) of contrasting maize cultivars at selected levels of Al stress under sand culture.

Table 10
Al & P accumulation of contrasting maize cultivars grown for 40 days at different concentration of aluminium under sand culture.

Category	Cultivar	Root Al (mg/g)				Shoot Al (mg/g)				Root P (%)				Shoot P (%)			
	Al (μM)	0	150	250	Mean	0	150	250	Mean	0	150	250	Mean	0	150	250	Mean
Tolerant	**Megha-9**	0.008	0.482	0.517	**0.336**	0.002	0.158	0.023	0.061	0.287	0.440	0.444	0.391	0.507	0.339	0.595	0.481
	Megha-10	0.002	0.330	0.372	**0.235**	0.001	0.148	0.094	0.081	0.685	0.752	0.771	0.736	0.915	0.488	0.831	0.745
	Mizo-19	0.003	0.279	0.317	**0.200**	0.001	0.149	0.088	0.080	0.782	0.782	0.782	0.782	0.904	1.033	0.735	0.891
	Mean	**0.004**	**0.364**	**0.402**	0.257	0.002	0.152	**0.068**	**0.074**	**0.585**	**0.658**	**0.666**	**0.636**	**0.776**	**0.620**	**0.720**	**0.705**
Moderately Tolerant	**Megha-1**	0.001	0.402	0.426	**0.276**	0.002	0.262	0.211	0.159	0.350	0.376	0.359	0.361	0.677	0.476	0.729	0.627
	Mizo-22	0.001	0.415	0.471	**0.296**	0.003	0.277	0.281	0.187	0.319	0.345	0.365	0.343	0.668	0.570	0.467	0.568
	Mizo-42	0.004	0.514	0.245	**0.254**	0.004	0.112	0.169	0.095	0.493	0.463	0.506	0.488	0.673	0.668	0.667	0.670
	Mean	0.002	**0.444**	**0.381**	**0.276**	**0.003**	**0.217**	**0.221**	**0.147**	**0.387**	**0.395**	**0.410**	**0.397**	**0.673**	**0.571**	**0.621**	**0.622**
Sensitive	**BRL Local**	0.008	0.108	0.095	**0.070**	0.002	0.138	0.169	0.103	0.348	0.338	0.351	0.345	1.069	0.490	0.980	0.846
	Sublgarh	0.002	0.261	0.440	**0.235**	0.003	0.324	0.206	0.178	1.031	0.945	0.948	0.975	1.158	0.972	0.933	1.021
	Uruapara	0.005	0.114	0.496	**0.205**	0.001	0.134	0.222	0.119	0.494	0.484	0.461	0.480	0.561	0.631	1.061	0.751
	Mean	**0.005**	**0.161**	**0.344**	**0.170**	0.002	**0.198**	**0.199**	**0.133**	**0.624**	**0.589**	**0.587**	**0.600**	**0.929**	**0.697**	**0.991**	**0.873**
Check	**RCM-76**	0.002	0.560	0.519	**0.360**	**0.003**	0.310	0.228	0.180	0.686	0.662	0.642	0.664	0.985	0.943	0.666	0.865
Factors		**C.D**	**SE(d)**	**SE(m)**	**F.V**	**C.D**	**SE(d)**	**SE(m)**	**F.V**	**C.D**	**SE(d)**	**SE(m)**	**F.V**	**C.D**	**SE(d)**	**SE(m)**	**F.V**
Factor (A)		**0.031**	0.015	0.011	**55*****	0.015	0.007	0.005	83***	0.130	0.065	0.046	44***	0.130	0.065	0.046	12***
Factor (B)		**0.017**	0.008	0.006	**12*****	0.008	0.004	0.003	14***	0.071	0.035	0.025	0.5NS	0.071	0.035	0.025	9.5***
Factor (A*B)		**0.054**	0.027	0.019	**33*****	0.025	0.013	0.009	37***	0.224	0.112	0.079	0.4NS	0.224	0.112	0.079	4.8***

Note. A –Maize cultivar; B –Al stress level; F.V– F value; NS = not significant.

in doses of Al while it declined to 5.9 % and 6.4 % sensitive cultivars and check variety whereas shoot P content of tolerant and moderately tolerant cultivars has declined by 7.2 % and 7.7 %, respectively. Fig. 6A depicts that the P utilization efficiency of tolerant maize cultivars was found to be 89.3 % and 39.8 % higher than the sensitive cultivars and check variety. P utilization efficiency of tolerant and moderately tolerant cultivars increased to the extent of 74.6 % and 44.9 % while it decreased in sensitive cultivars and check variety (16.6 % and 32.9 %) with the increase in Al concentration. Similarly, Fig. 6B shows that the P uptake efficiency of tolerant cultivars was highest although it gradually decreased with increase in Al stress.

3.4. Validation of Al stress response of selected maize lines

Nine genotypes selected based on scoring on the response physio-morphological traits (Supplementary Table 2A) was evaluated for Al toxicity tolerance in acid soil. The pot experiment with soil (Fig. 7A) revealed that tolerant cultivar MZM-19 recorded 69 % higher total chl (1.33 mg/g) than sensitive cultivar, *Sublgarh* bearing lowest total chl of 0.41 mg/g (Supplementary Table 8). Supplementary Table 8 revealed that marked differences were observed in the mean values for each parameter such as chl content, carotenoids, anthocyanin, and cell membrane stability (CMS) among three different classes of genetypes. Tolerant cultivars, exemplified by Megha-9, Megha-10, and MZM-19, exhibited higher chl levels compared to moderately tolerant and sensitive cultivars. Additionally, the check variety, RCM-76, demonstrated intermediate values for chl content relative to other cultivars. The mean values for chl *a*, chl *b*, and total chl are 0.54 mg/g, 0.47 mg/g, and 0.99 mg/g, respectively, across all cultivars. Higher chl *a* and *b* (0.66 mg/g and 0.71 mg/g) was recorded by tolerant MZM-19 over the sensitive cultivar *Sublgarh* (0.27 and 0.14 mg/g) to the tune 59.1 % and 80.2 %. The chl *a* to b ratio averages at 1.14 with sensitive cultivars recording the highest. Carotenoid levels range from 14.80 μg/g to 44.99 μg/g, while anthocyanin levels range from 15.68 μg/g to 336.28 μg/g. The genetic variability percentages indicate the extent of variability within the dataset attributed to genetic factors, with values ranging from 9.708 % to 28.14 % for the parameters evaluated. Sensitive cultivar *Sublgarh* recorded the highest SLW of 3.33 mg/cm^2 whereas moderately tolerant cultivar MZM-42 had the lower specific leaf weight of 1.39 mg/cm^2 which ideally reflects the changes in leaf thickness.

Tolerant cultivars show higher TRL, RSA, and RV compared to moderately tolerant and sensitive cultivars. For instance, Megha-9 exhibits the highest TRL (876.90 cm), RSA (491.44 cm^2), and RV (21.57 cm^3) among all cultivars. Conversely, Sensitive cultivars like BRL Para, Sublgarh, and Uruapara demonstrate lower values for these parameters. The check variety, RCM-76, falls within intermediate ranges for root morphology parameters (Supplementary Table 9). In case of rhizosphere acidification, the intensity of colour change was more in tolerant and moderate cultivars compared to sensitive cultivars and check variety (Supplementary Fig. 4). Furthermore, tolerant cultivars exhibits higher root and shoot dry weights compared to moderately tolerant and sensitive cultivars. Specifically, Megha-9 demonstrates the highest RDW (0.48 g) and SDW (0.63 g) among all cultivars in this category (Supplementary Table 10). Moderately tolerant cultivars exhibits intermediate values for RDW and SDW with Megha-1. Meanwhile, sensitive cultivars

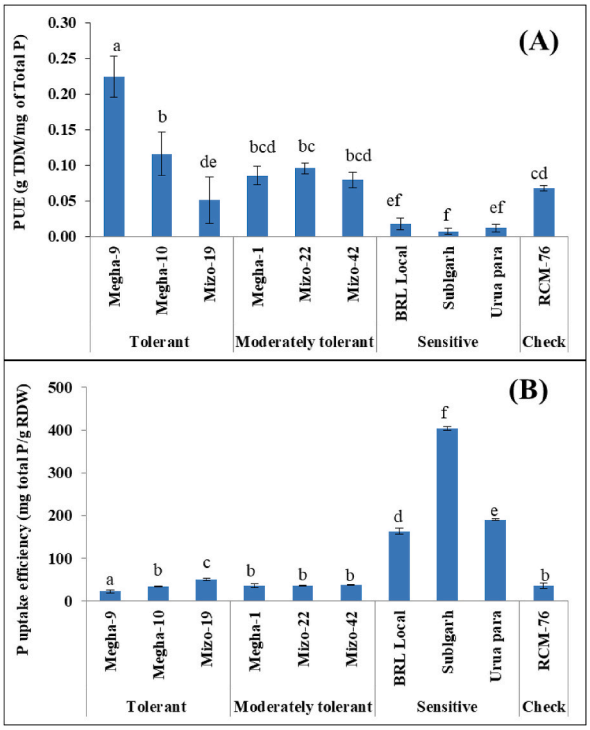

Fig. 6. P utilization efficiency (A) and P uptake efficiency (B) of identified lines of maize exposed to selected levels of Al stress under sand culture.

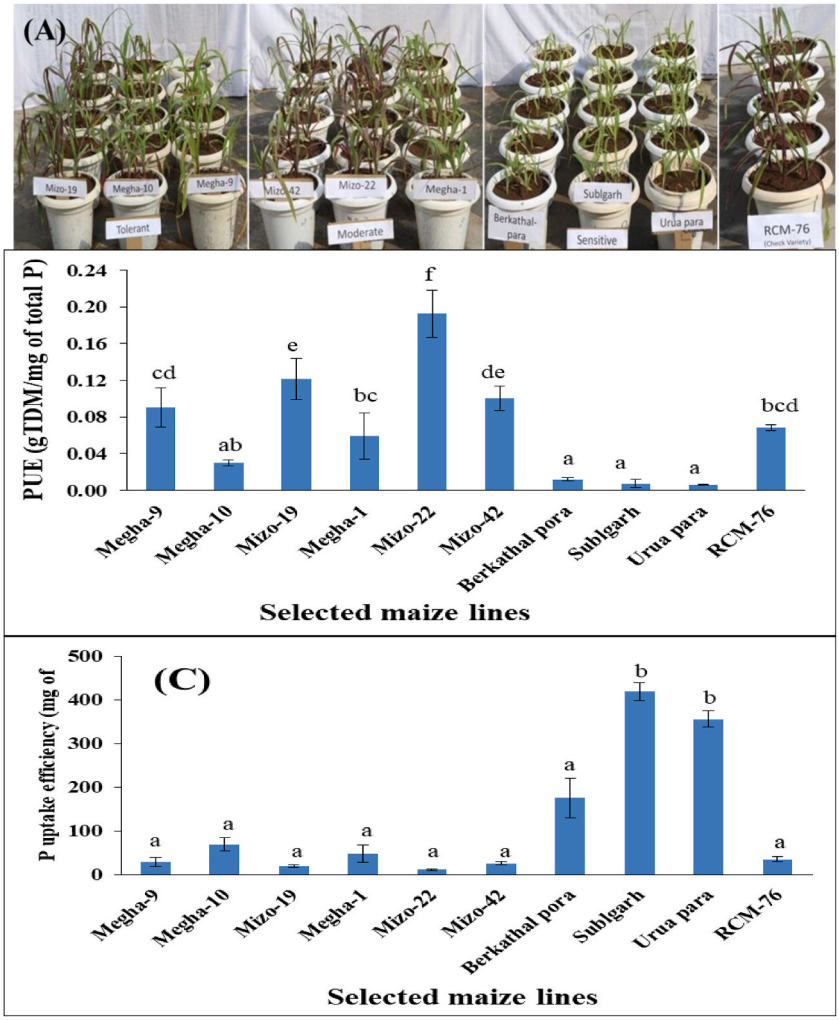

Fig. 7. Microcosm experiment with acid soil collected from field (A) Phosphorus utilization efficiency (B) and Phosphorus Uptake efficiency (C) of selected maize cultivars grown in acid soil.

have lowest RDW and SDW compared to tolerant and moderately tolerant cultivars, indicating reduced biomass accumulation under stress conditions. The genetic variability percentages indicate the extent of variability within the dataset attributed to genetic factors, with values ranging from 18.6 % to 27.4 % for the parameters evaluated. Tolerant cultivar Megha-10 had the highest concentration of Al in root (1.65 mg/g) and shoot (0.39 mg/g) over sensitive cultivar BRL Para (0.70 mg/g and 0.18 mg/g) amounting to the difference of 42.6 % and 11 % (Supplementary Fig. 5). Sensitive cultivar *Uruapara* and *Sublgarh* had the highest root P (1.43 %) and highest shoot P (1.60 %) respectively whereas moderately tolerant cultivar MZM-22 and MZM-42 had the lowest root P (0.24 %) and least shoot P (0.13 %) respectively (Supplementary Fig. 6). Moderately tolerant cultivar *Sublgarh* had the highest PUE of 0.19 g TDM/mg of shoot P whereas sensitive cultivar *Uruapara* had the least PUE of 0.006 g TDM/mg of shoot P but showed highest P uptake efficiency of 341.5 mg of P/g of RDW over tolerant cultivar Mizo-19 which had the least P uptake efficiency of 19.94 of P/g of RDW. Moderately tolerant cultivars have significantly higher PUE and lower uptake efficiency as compared to the sensitive cultivars (Fig. 7B and 7BCE). The observed variations in Al stress responses among cultivars under aluminum toxicity and their differing root system characteristics imply diverse adaptations to predominant edaphic stress in the region. These differences provide insights into their adaptability and growth potential under challenging stress conditions.

4. Discussion

4.1. Physio-morphological traits are key stress indicators of Al toxicity stress

The present study conducted using sand and soil culture provided an opportunity to assess the impact of aluminum (Al) stress on maize at both root and shoot levels. Most of the root growth parameters exhibited a gradual reduction with increasing levels of Al.

However, the degree of reduction till 150 μM was relatively less compared to the significant reduction observed at 300 μM Al. The accumulation of Al and extent of root cell death increased with higher concentrations of Al in the growing medium, as evidenced by the intensity of blue coloration resulting from the interaction between hematoxylin stain and absorbed Al in the exposed root area. Similar findings were observed in barley seedlings, where increased Al concentration in the soil lead to the increased sensitivity towards plants growth parameters [39]. The uneven and radial expansion of cortex cells leads to root thickening and mechanical stress on the epidermis [40]. Earlier studies have revealed that plants utilize diverse strategies to combat Al-induced phytotoxicity, including reducing the uptake of Al^{3+} into the roots and translocating it to above-ground tissues, enhancing chelation and sequestration mechanisms, and bolstering the antioxidant capacity of the plant [41,42]. The qualitative agar medium assay used in this study indicated differential rhizosphere acidification, consistent with reports (17,30,31).

Higher level of Al in the tissues leads to decline in chlorophyll content thereby reducing photosynthesis rate which is evident in our current study with significant reduction in Chl a, Chl b, total chl and carotenoid content with increasing dose of Al. This decline in chlorophyll content may be attributed to accelerated breakdown of chlorophyll due to elevated sensitivity or activation of chlorophyllase or peroxidase enzyme [43,44], distortion in the chloroplast architecture, reduction of electron transport in photo system II (PS II) [45] and also possibly due to increased, Mg deficiency caused by Al toxicity thereby reducing chlorophyll synthesis [46]. However, anthocyanin being a stress responsive pigment is synthesized to protect the plant from high concentration of Al. Short term exposure of maize seedlings to mild Al stress seems to be harmless as it only increased leaf pigment. Similar study was conducted in potato showing enhancements of Chl a, Chl b, total Chl to 21–35 % and carotenoids to 12.4% when potatoes were exposed to 50 μM Al [47,48]. Rise of even 1 % in leakage upon Al treatment has greater influence on CMS and membrane function during plant metabolism (31).

The presence of Al in the shoot showed an inverse correlation with both LDW and photosynthetic rate. A significant decline in shoot parameters was observed in plants grown in a medium with Al concentration above 150 μM. This decline could be attributed to several factors, including reduced root absorbing area, diminished photosynthetic leaf area, and subsequently reduced nutrient supply (P, Ca, Mg etc.). Additionally, poor root growth and limited water supply resulted in stunted shoot growth [49]. The highest root-to-shoot ratio was observed under mild Al stress (50 μM Al). Furthermore, our investigation revealed a strong inverse correlation between root Al concentration and both TRL ($r^2 = 0.797$) and RSA ($r^2 = 0.773$), indicating the detrimental effect of increased Al concentration on these root parameters (Fig. 8A and B). The most commonly observed response to Al-induced shoot growth inhibition involves alterations in cellular and ultra-structures in leaves, reduction in stomatal aperture, decreased CO_2 entry, diminished photosynthetic activity, as well as chlorosis and necrosis of leaves [45]. Current investigation revealed that Al content in root and shoot tissues gradually increased with the increased levels of Al. Also, shoot to root Al ratio increased with increasing dose of Al till 100 μM beyond which the ratio declined sharply which indicates that higher amount of Al was translocated to shoot up to 100 μM. Higher accumulation of Al in the roots in comparison to shoot might be due to formation of ligand complexes in the apoplast, rhizosphere acidification to diminish the activity of Al^{3+} ions near the root surface, or minimization the capacity of cells to transport Al [39,50] and thereby reducing root activity and nutrient mobilization capacity. However, phosphorus accumulation in root and shoot was observed to be the maximum at 200 μM Al which indicates that external application of Al enhances P uptake by maize plants. This finding was in accordance with the study conducted on rice and barley [51]. Precipitation of Al–P complex is responsible for the elevated level of P in root tissues [52]. However, P utilization efficiency declined with the increase in Al dose which caused due to formation of Al–P precipitate which in turn reduces P translocation from roots to shoot. Therefore, this study showed that 150 μM is tolerable limit of Al stress and 250 μM is toxic level of Al stress under sand and soil culture assay.

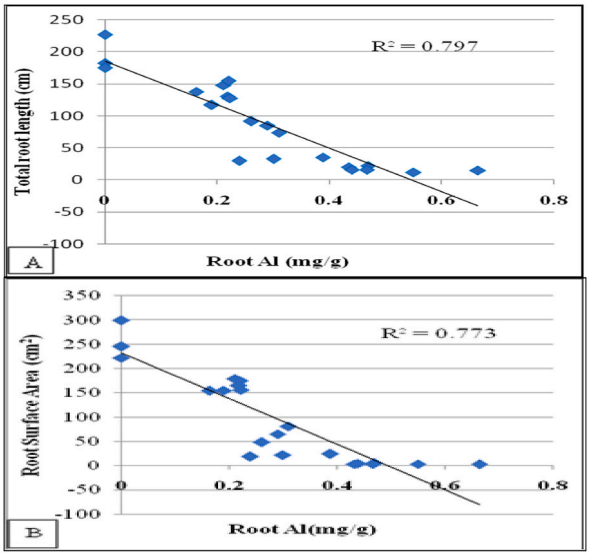

Fig. 8. Relationship between root Al contents with root parameters (A and B).

4.2. Genotypic variability of differential Al stress tolerance in maize

Better performance of the tolerant cultivars in comparison to sensitive cultivars in most of root parameters under Al toxicity demonstrates tolerance capacity of maize cultivars. Similar study was performed by Foy et al. [53] and Richard et al. [54] to assess genotypic variation of fourteen maize inbreds towards Al toxicity through hydroponic assay. Megh-9 genotype is tolerant towards Al stress as it had significantly higher root length, root volume, root surface area, and root diameter over sensitive genotype like *sublgarh* and MZM-42. In terms of shoot parameters tolerant cultivars were recorded higher leaf pigments (i.e., chl *a*, chl *b*, carotenoids and anthocyanin), leaf area, leaf perimeter, and specific leaf weight over sensitive cultivars. Although, the Chl a/b ratio, carotenoids to total chl and anthocyanin to total chl diminished in tolerant cultivars there was a remarkable increase in CMS and shoot length in tolerant genotypes. Besides this, tolerant genotypes had higher photosynthetic rate and reduced transpiration indicating the sensitivity of transpiration for Al stress. Tolerant cultivar like Megh-9 and Megh 11 were recorded with highest biomass and increased shoot P accumulation which may be attributed to elevated rate of photosynthesis under stress. The decline in photosynthetic activity as observed in the sensitive cultivars of highbush blueberry [55], Eucalyptus [48] and beech seedlings [56] as induced by Al stress. Acid and Al stress exerted negative effect on photosynthetic activity of eucalyptus [48]. Reduced leaf thickness and leaf morphology was evident in the study as Al stress intensity has increased. Similar type of leaf structural changes was reported in eucalyptus by Yang et al. [48].

The concentration of Al in shoot tissue of tolerant cultivars was significantly lower to the tune of 79.7 % compared to that of moderate and sensitive cultivars which may be attributed to the restriction of Al entry via formation of chelates with organic acids as demonstrated with the rhizosphere acidification assay. Under high Al level stress (250 μM); tolerant cultivar has maintained higher phosphorus accumulation in shoot than root to rescue the disturbed metabolism in the shoot [57]. Since various studies has established that tolerant cultivars have capability to secrete more organic acids which may lead to chelation of Al at plasma membrane and thereby prevents its uptake whereas susceptible cultivars have allowed more Al entry and get affected with more root damage [19,58]. Further, it was interesting to observe that P utilization efficiency was found higher whereas P uptake efficiency was found lower in tolerant cultivars compared to the sensitive cultivars and check variety. Al toxicity is reported to closely associate with P nutrition as it could be effective agent to detoxify Al [59].

4.3. Sand culture is promising screening tool for large scale Al stress profiling

To examine degree of changes and consistency of the results obtained in sand culture, validation experiment in acid soil was conducted. The root exudation was distinct and better than that observed in sand culture assay. The clear and visible expression of root exudation pattern in soil may be due to combined effect of Al toxicity and unavailability of phosphorus that stimulated the plant to exude more organic acids in order to adapt to acidic soil (pH 3.92) conditions. Since exudation was more in tolerant cultivars, they have better adaptability. The trends of root exudation remained the same in soil which illustrates tolerant cultivars have capability to exude more organic acids than moderate and sensitive cultivars. The performance of selected cultivars with respect to root morphological traits, shoot growth characteristics, biomass characteristics and tissue Al and P accumulation was similar and followed the same trends as observed in case of sand culture assay. The extent of anthocyanin synthesis was also clearly demarcated among the contrasting cultivars. Tolerant and moderately tolerant cultivars exhibited higher capability to synthesize more anthocyanin in the leaves compared to sensitive ones. Moreover, the growth habit and biomass accumulation of tolerant and moderately tolerant cultivars was found to be robust and vigorous compared to sensitive ones. According to Mapiemfu-Lamare et al. [60] the tolerance capacity of the maize cultivars towards Al toxicity is due to the production of organic and phenolic compounds by plants and soil type.

Thus this study revealed that physio-morphological traits viz., A, RV, RSA, anthocyanin and Chlorophyll *b*, total chlorophyll and TDM were emanated as key traits for assessing Al toxicity stress tolerance in Maize with high divergence values which could be of potential utility for large scale Al stress screening. Current study identifies Al concentration of 150 μM as critical limit for maize seedling and maize cultivars viz. Megh-9, Megh10 and MZM-19 as tolerant, Megh-1, MZM-22 and MZM-42 as moderately tolerant and BRL para, *Subhalgarh* and *Uruapara* as sensitive to Al toxicity. Revalidation of these identified cultivars under acid soil validates at par Al stress response implying greater utility of selected Al stress tolerating maize cultivars for future maize improvement under challenging agro-ecologies of EHR with extensive soil acidity.

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Ethics approval

Not applicable.

Consent to participate

Not applicable.

Consent to publish

All authors approved and consented to publish this work.

Data availability statement

The datasets presented in this study will be provided on request.

CRediT authorship contribution statement

Naresh Bhukya: Writing – original draft, Resources, Methodology, Investigation, Data curation. **Samarendra Hazarika:** Writing – review & editing, Supervision, Project administration, Investigation, Conceptualization. **Krishnappa Rangappa:** Writing – original draft, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Dwipendra Thakuria:** Writing – review & editing, Resources, Methodology, Investigation, Conceptualization. **Rumi Narzari:** Writing – review & editing, Validation, Formal analysis. **Supriya Debnath:** Writing – review & editing, Resources, Formal analysis, Data curation.

Declaration of competing interest

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Appendix A. Supplementary data

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References

[1] A. Kumar, R.P.S. Verma, A. Singh, H.K. Sharma, G. Devi, Barley landraces: Ecological heritage for edaphic stress adaptations and sustainable production, Environmental and Sustainability Indicators 6 (2020) 100035.
[2] U.C. Sharma, R.P. Singh, Acid Soils of India: Their Distribution, Management and Future Strategies for Higher Productivity, 2002.
[3] S. Hazarika, A. Nabam, D. Thakuria, S. Kataki, R. Krishnappa, Lime equivalence of organic manures and scope of their utilization as acid soil amendments, Arch. Agron Soil Sci. 67 (5) (2021) 660–674.
[4] M.A. Rahman, S.H. Lee, H.C. Ji, A.H. Kabir, C.S. Jones, K.W. Lee, Importance of mineral nutrition for mitigating aluminum toxicity in plants on acidic soils: current status and opportunities, Int. J. Mol. Sci. 19 (10) (2018) 3073.
[5] S. Hazarika, B. Sohliya, D. Thakuria, S. Kataki, K. Rangappa, Influence of organic amendments on acidic soil responsive crop groundnut (Arachishypogaea L.), Environ. Prog. Sustain. Energy 40 (4) (2021) e13592.
[6] Manoj-Kumar, North East India: soil and water management imperatives for food security in a changing climate, Curr. Sci. 101 (2011) 1119.
[7] Manoj-Kumar, Evidences, projections and potential impacts of climate change on food production in North East India, Indian Journal of Hill Farming 24 (2011) 1–10.
[8] A. Das, J. Layek, R.G. Idapuganti, S. Basavaraj, R. Lal, K. Rangappa, S. Ngachan, Conservation tillage and residue management improves soil properties under a upland rice–rapeseed system in the subtropical eastern Himalayas, Land Degrad. Dev. 31 (14) (2020) 1775–1791.
[9] Z.B. Yang, W.J. Horst, Aluminum-induced inhibition of root growth: roles of cell wall assembly, structure, and function, Aluminum stress adaptation in plants (2015) 253–274.
[10] Y.F. Niu, R.S. Chai, G.L. Jin, H. Wang, C.X. Tang, Y.S. Zhang, Responses of root architecture development to low phosphorus availability: a review, Annals of botany 112 (2) (2013) 391–408.
[11] C. Poschenrieder, B. Gunsé, I. Corrales, J. Barceló, A glance into aluminum toxicity and resistance in plants, Science of the total environment 400 (1–3) (2008) 356–368.
[12] J. Barcelo, C. Poschenrieder, Fast root growth responses, root exudates, and internal detoxification as clues to the mechanisms of aluminium toxicity and resistance: a review, Environ. Exp. Bot. 48 (1) (2002) 75–92.
[13] S.A. Bhalerao, D.V. Prabhu, Aluminium toxicity in plants—a review, Journal of Applicable Chemistry 2 (3) (2013) 447–474.
[14] E. Delhaize, P.R. Ryan, P.J. Randall, Aluminum tolerance in wheat (Triticumaestivum L.)(II. Aluminum-stimulated excretion of malic acid from root apices), Plant physiology 103 (3) (1993) 695–702.
[15] Y. Yamamoto, Y. Kobayashi, H. Matsumoto, Lipid peroxidation is an early symptom triggered by aluminum, but not the primary cause of elongation inhibition in pea roots, Plant physiology 125 (1) (2001) 199–208.
[16] J.F. Ma, P. Ryan, E. Delhaize, Aluminium tolerance in plants and the complexing role of organic acids, Trends Plant Sci. 6 (6) (2000) 273–278.

[17] R. Krishnappa, I.S. Aftab Hussain, Phosphorus acquisition from deficient soil: involvement of organic acids and acid phosphatase in pigeon pea (*Cajanuscajan L.* mills sp), Indian J. Plant Physiol. 19 (2014) 197–204.
[18] M.T. Iqbal, Phosphorus enhances aluminium tolerance in both aluminium-tolerant and aluminium-sensitive wheat seedlings, S. Afr. J. Plant Soil 30 (1) (2013) 13–21.
[19] M.T. Iqbal, *The effects of Aluminium-Phosphorus Interactions on Plant Growth in Acidic Soils* (Doctoral Dissertation, La Trobe), 2011.
[20] S.J. Zheng, J.L. Yang, Y.F. He, X.H. Yu, L. Zhang, J.F. You, H. Matsumoto, Immobilization of aluminum with phosphorus in roots is associated with high aluminum resistance in buckwheat, Plant Physiology 138 (1) (2005) 297–303.
[21] K. Tan, W.G. Keltjens, Interaction between aluminium and phosphorus in sorghum plants: I. Studies with the aluminium sensitive sorghum genotype TAM428, Plant Soil 124 (1990) 15–23.
[22] V. Kumar, S. Singh, R.S. Chhokar, R.K. Malik, D.C. Brainard, J.K. Ladha, Weed management strategies to reduce herbicide use in zero-till rice–wheat cropping systems of the Indo-Gangetic Plains, Weed Technol. 27 (1) (2013) 241–254.
[23] J.F. Ma, S.J. Zheng, X.F. Li, K. Takeda, H. Matsumoto, A rapid hydroponic screening for aluminium tolerance in barley, Plant Soil 191 (1997) 133–137.
[24] T. Sasaki, Y. Yamamoto, B. Ezaki, M. Katsuhara, S.J. Ahn, P.R. Ryan, H. Matsumoto, A wheat gene encoding an aluminum-activated malate transporter, Plant J. 37 (5) (2004) 645–653.
[25] J.V. Magalhaes, J. Liu, C.T. Guimaraes, U.G. Lana, V.M. Alves, Y.H. Wang, L.V. Kochian, A gene in the multidrug and toxic compound extrusion (MATE) family confers aluminum tolerance in sorghum, Nat. Genet. 39 (9) (2007) 1156–1161.
[26] A.N. Famoso, R.T. Clark, J.E. Shaff, E. Craft, S.R. McCouch, L.V. Kochian, Development of a novel aluminum tolerance phenotyping platform used for comparisons of cereal aluminum tolerance and investigations into rice aluminum tolerance mechanisms, Plant physiology 153 (4) (2010) 1678–1691.
[27] O.J. Garcia, W.D. Silva, M.A. Massei, An efficient method for screening maize inbreds for aluminium tolerance, Maydica 24 (1979) 75–82.
[28] T.C. Baruah, H.P. Borthakur, Soil Chemistry. Baruah, TC, Borthakur, HP, A Textbook of Soil Analysis, Vikas Publishing House Pvt. Ltd., New Delhi, 1997, pp. 118–132.
[29] M. Misyura, J. Colasanti, S.J. Rothstein, Physiological and genetic analysis of Arabidopsis thaliana anthocyanin biosynthesis mutants under chronic adverse environmental conditions, J. Exp. Bot. 64 (1) (2013) 229–240.
[30] A. Das, K. Rangappa, S. Basavaraj, U. Dey, M. Haloi, J. Layek, S. Ngachan, Conservation tillage and nutrient management practices in summer rice (Oryza sativa L.) favoured root growth and phenotypic plasticity of succeeding winter pea (PisumsativumL.) under eastern Himalayas, India, Heliyon 7 (5) (2021) e07078.
[31] K. Hazarika, K.R. Goswami, B. Bharali, P. Kalita, J. Goswami, B. Neog, Physiological performance of sweet sorghum (Sorghum bicolor L. Moench) genotypes under Assam situation, Research on Crops 23 (3) (2022) 556–561.
[32] M.L. Jackson, Soil Chemical Analysis, Prentice Hall of India (pvt.) Ltd., New Delhi, 1973.
[33] V. Romheld, C. Muller, H. Marschner, Localization and capacity of proton pumps in roots of intact sunflower plants, Plant Physiology 76 (3) (1984) 603–606.
[34] E.K.C.F. Polle, C.F. Konzak, J.A. Kattrick, Visual detection of aluminum tolerance levels in wheat by hematoxylin staining of seedling roots 1, Crop Sci. 18 (5) (1978) 823–827.
[35] R.A. Rangaswamy, Text Book of agricultural Statistics, in: New Age International Publishers, Wiley Eastern, New Delhi, 1995.
[36] K.A. Gomez, A.A. Gomez, Statistical Procedures for Agricultural Research, John wiley & sons, New York, 1984.
[37] O.P. Sheoran, D.S. Tonk, L.S. Kaushik, R.C. Hasija, R.S. Pannu, Statistical software package for agricultural research workers, in: Recent Advances in Information Theory, Statistics & Computer Applications by DS Hooda& RC Hasija Department of Mathematics Statistics, CCS HAU, Hisar, vol. 8, 1998, pp. 139–143, 12.
[38] V. Odedra, N. Karmakar, T. Vyas, M.K. Debnath, K. Patel, P. Faldu, Biochemical screening and Fatty acid Profiling of Niger seeds (guizotiaabyssinica) grown in India, Proc. Natl. Acad. Sci. India B Biol. Sci. 94 (2) (2024) 261–269.
[39] M.F. Dawood, M. Tahjib-Ul-Arif, A.A.M. Sohag, A.A.H. Abdel Latef, Fluoride mitigates aluminum-toxicity in barley: morpho-physiological responses and biochemical mechanisms, BMC Plant Biol. 22 (1) (2022) 1–17.
[40] M. Čiamporová, Diverse responses of root cell structure to aluminium stress, Plant Soil 226 (1) (2000) 113–116.
[41] A. Emamverdian, Y. Ding, F. Mokhberdoran, Y. Xie, Heavy metal stress and some mechanisms of plant defense response, Sci. World J. 2015 (2015).
[42] R. Rahman, H. Upadhyaya, Aluminium toxicity and its tolerance in plant: a review, J. Plant Biol. 64 (2021) 101–121.
[43] S. Ali, F. Zeng, L. Qiu, G. Zhang, The effect of chromium and aluminum on growth, root morphology, photosynthetic parameters and transpiration of the two barley cultivars, Biol. Plantarum 55 (2011) 291–296.
[44] T. Houri, Y. Khairallah, A. Al Zahab, B. Osta, D. Romanos, G. Haddad, Heavy metals accumulation effects on the photosynthetic performance of geophytes in Mediterranean reserve, J. King Saud Univ. Sci. 32 (1) (2020) 874–880.
[45] R. Ofoe, R.H. Thomas, S.K. Asiedu, G. Wang-Pruski, B. Fofana, L. Abbey, Aluminum in plant: benefits, toxicity and tolerance mechanisms, Front. Plant Sci. 13 (2023) 1085998.
[46] Z.C. Chen, J.F. Ma, Magnesium transporters and their role in Al tolerance in plants, Plant Soil 368 (2013) 51–56.
[47] B. Lazarević, V. Jurkić, M. Musić, M. Poljak, Effect of aluminium toxicity on concentration of photosynthetic pigments in two potato cultivars with different aluminium sensitivity, VI Balkan Symposium on Vegetables and Potatoes 1142 (2014) 61–66.
[48] M. Yang, L. Tan, Y. Xu, Y. Zhao, F. Cheng, S. Ye, W. Jiang, Effect of low pH and aluminum toxicity on the photosynthetic characteristics of different fast-growing Eucalyptus vegetatively propagated clones, PLoS One 10 (6) (2015) e0130963.
[49] S.K. Panda, F. Baluška, H. Matsumoto, Aluminum stress signaling in plants, Plant Signal. Behav. 4 (7) (2009) 592–597.
[50] D.L. Jones, P.R. Ryan, NUTRITION: aluminum toxicity, in: B. Thomas (Ed.), Encyclopedia of Applied Plant Sciences, Elsevier, 2003, pp. 656–664.
[51] S. Ishikawa, T. Wagatsuma, R. Sasaki, P. Ofei-Manu, Comparison of the amount of citric and malic acids in Al media of seven plant species and two cultivars each in five plant species, Soil Sci. Plant Nutr. 46 (3) (2000) 751–758.
[52] L.H. McCormick, F.Y. Borden, Phosphate fixation by aluminum in plant roots, Soil Sci. Soc. Am. J. 36 (5) (1972) 799–802.
[53] C.D. Foy, R.T. Chaney, M.C. White, The physiology of metal toxicity in plants, Annu. Rev. Plant Physiol. 29 (1) (1978) 511–566.
[54] C.A. Richard, L.T. Hickey, S. Fletcher, R. Jennings, K. Chenu, J.T. Christopher, High-throughput phenotyping of seminal root traits in wheat, Plant Methods 11 (1) (2015) 1–11.
[55] M.P. Cárcamo, M. Reyes-Díaz, Z. Rengel, M. Alberdi, R.P. Omena-Garcia, A. Nunes-Nesi, C. Inostroza-Blancheteau, Aluminum stress differentially affects physiological performance and metabolic compounds in cultivars of highbush blueberry, Sci. Rep. 9 (1) (2019) 11275.
[56] M. Ridolfi, J.P. Garrec, Consequences of an excess Al and a deficiency in Ca and Mg for stomatal functioning and net carbon assimilation of beech leaves, Ann. For. Sci. 57 (3) (2000) 209–218.
[57] W. Teng, Y. Kang, W. Hou, H. Hu, W. Luo, J. Wei, B. Zhang, Phosphorus application reduces aluminum toxicity in two Eucalyptus clones by increasing its accumulation in roots and decreasing its content in leaves, PLoS One 13 (1) (2018) e0190900.
[58] E. Delhaize, P.R. Ryan, Aluminum toxicity and tolerance in plants, Plant Physiol. 107 (2) (1995) 315.
[59] E.G. Bollard. Involvement of unusual elements in plant growth and nutrition, Encyclopedia of plant physiology. New series, Springer, 1983, pp. 695–744.
[60] D. Mapiemfu-Lamaré, S.A. Ndindeng, A.F. Ngome, C. Thé, E. Tsoata, C. Zonkeng, F. Etame, Early criterion to screen maize varieties for their tolerance to aluminium toxic soil, Int. J. Agric. For. 2 (4) (2012) 161–165.