PHYTOTOXIC EFFECTS OF ALUMINIUM ON GROWTH AND METABOLISM OF PISUM SATIVUM L.

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ABSTRACT

In the present study, the morphological and biochemical characteristics of two pea (Pisum sativum) varieties Arkil and Rachana were evaluated in response to aluminium (Al) stress in soil. Soil was treated with 0.2, 0.4, 0.6 and 0.8 g/kg of Al. Observations were made on seed germination, seedling growth, pigment, protein and sugar content. Lipid peroxidation (LP) was also measured. Several antioxidant enzymes activities such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), guaicol peroxidase (GPX), ascorbic acid (AsA) and non protein thiol (NPSH) content were investigated against oxidative stress caused by Al. Seed germination and seedling growth of both the varieties remarkably reduced as compared to control. LP was significantly ($p<0.05$) increased in the seedling of the two varieties, especially in the sensitive one. In the presence of Al, antioxidant enzymes activities markedly ($p<0.01$) enhanced in both varieties but it increased more prominently in Rachana variety. So, Rachana variety probably accumulated fewer amounts of reactive oxygen species and other toxic phenolic compounds and, consequently grows better under Al toxicity. The results of the present study may help in understanding the mechanism involved against oxidative stress and their possible use in phytoremediation.

Key words: Aluminium, antioxidant enzymes, ascorbic acid, non-protein thiol and lipid peroxidation.

INTRODUCTION

Ecological impact assessment of contamination stress on plants has been an interesting area of research in the last few years. Metals are among the major contaminants found in both contaminated and natural soils. Aluminium (Al) is a widespread and most abundant metal. It is a light metal and makes up 7% of the earth's crust. Aluminium is one of the major growth limiting factors in acid soil throughout the world. Application of ammonium ion producing fertilizers (e.g. urea, anhydrous ammonia, ammonium sulphate) acidify soil through a biological reaction by which ammonium ion is oxidized to nitrate ion and hydrogen ion. Sulfur dioxide (SO_2) and nitrogen oxide (NO_X) released primarily by industrial activities react with water to form acid rain, which also acidifies the soils. If soil becomes acidic, Al is solubilized into toxic form generally referred to Al^{3+}. Al toxicity generally interferes with cell division in root tips and inhibits root elongation by increasing cell wall rigidity by cross linking pectins[1]. It has been reported that plasma membrane is the major target for Al toxicity due to the presence of negative charges on carboxyl group and phosphate group in plasma membrane. A common feature of Al toxicity was noticed to enhance the production of reactive oxygen species (ROS), like superoxide radical ($O_2^{\cdot-}$), hydroxyl radical ($^{\cdot}OH$), alkoxy radical ($^{\cdot}RO$), singlet oxygen ($^1O_2$), and toxic hydrogen peroxide (H_2O_2) molecules[2,3]. These ROS causes harmful effect on the plant cells[4]. An enhancement in the activity of superoxide dismutase and peroxidase represents the presence of an antioxidant scavenging system in Al-treated roots[5]. Al stress also induced an elevation on lipid peroxidation [6]. Considerable research has now been carried out to demonstrating the mechanism of Al toxicity and plant tolerance. Plant species and genotypes within species vary widely in tolerance against Al stress. Mechanism of Al tolerance has been broadly defined as those which prevent Al uptake by root and those which detoxify Al already accumulated in the cell [7]. Al tends to bind with carboxyl or phosphate groups more strongly as compared to –SH groups characteristic for cheatins[8]. However, Wu et al.[9], reported that

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plant metallothionein like protein and phytochelatins may play a major role in Al tolerance. The objective of this work was to evaluate the morphological and biochemical alteration in two varieties of *Pisum sativum* in response to Al stress.

MATERIALS AND METHODS

The certified seeds of *Pisum sativum* L. var. Arkil and Rachana were procured from Seed Agency at Allahabad. Healthy and uniform sized seeds of pea were surface sterilized with 0.001M $HgCl_2$ solution and then rinsed five times with distilled water. Subsequently the seeds were soaked in distilled water for 4 hours and sown in each experimental tray (9x9x1.5 inches) in four equidistant rows. Each tray was filled with 2.5 kg filtered soil. The soils were supplied with five aluminum concentrations treatments : Aluminum Chloride ($AlCl_3$): 0 g kg^{-1} soil (C), 0.2 g kg^{-1} soil (T1), 0.4 g kg^{-1} soil (T2), 0.6 g kg^{-1} soil (T3), 0.8 g kg^{-1} soil (T4) respectively. Three replicates (trays) with 16 plants in each tray were performed in each treatment. Plants were watered as when required. The seedling were maintained in a growth chamber under controlled temperature (20±2°C), photoperiod of 16/8 hrs and photon flux density of 240 µ mol m^{-2} s^{-1}.

Seed germination and seedling growth:

Germination was initiated after 2nd day of sowing. Germination up to 7 day at regular interval of 24hr was recorded. Seedling growth was recorded up to 15th day. Germination Rate (GR) and absolute growth rate (AGR) was calculated as follows:

GR = ∑ (number of seeds germinated) / (number of days)

$$AGR = (h_2 - h_1) / (t_2 - t_1) \text{ cm/day;}$$

where h_2 and h_1 are final and initial height of seedling; t_2 and t_1 are final and initial days.

First fully expanded leaves of 15 days old seedling were taken for biochemical analysis.

Determination of leaf photosynthetic pigment and protein content: Chlorophyll (a and b) and carotenoids were measured in fresh leaf samples. Leaf samples (10mg) were homogenized in 80% (v/v) acetone, filtered and then quantified spectrophotometrically according to Lichtenthaler[10]. Protein content was determined following the method of Lowry et al.[11] and amount of protein was calculated from standard curve obtained from bovine serum albumin.

Measurement of sugar content: Total soluble sugar was quantified according to Hedge and Hofreiter[12]. About 100mg of plant material were homogenized in 5ml of 95% ethanol and centrifuged. Supernatant (0.1 ml) was mixed with 4ml anthrone reagent and heated on boiling water bath for 10 minutes. Absorbance was taken at 620nm after cooling. Amount of sugar was calculated with reference to standard curve prepared from glucose.

Extraction and assay of enzymes: Plant material (500mg leaves) was homogenized in 0.1M phosphate buffer containing 1% polyvinylpyrrolidone (w/v) at 4°C. Homogenate was filtered through cheese cloth and centrifuged at 14,000*g* for 20 minutes. Supernatant was used to measure the enzymes activity.

Superoxide dismutase (1.15.11) activity was assayed by measuring its ability to inhibit photochemical reduction of nitroblue tetrazolium (NBT) according to Beyer and Fridovich[13]. The reaction mixture (3ml) contained 20mM methionine, 0.15mM Ethylene di-amine tetra acetic acid (EDTA), 0.12mM (NBT), 11.96µM Riboflavin and enzyme extract. Riboflavin was administered at the end. The test tube were shaken and kept for 30 minutes under 40W fluorescent lamp. The test tube containing enzyme kept in dark served as blank while the control tube without enzyme kept in light served as reference. The absorbance of solution was measured at 560nm. The activity expressed in enzymes unit g^{-1}FW.

Catalase (EC1.11.1.6) activity was assayed according to Cakmak and Marschner[14]. The reaction mixture (2ml) consisted of 1ml 25mM phosphate buffer (pH 7.0), 10mM H_2O_2 and 0.4ml enzyme extract. The activity was determined by measuring rate of disappearance of H_2O_2 for 1

minute at 240nm and calculated using extinction coefficient of 39.4$mM^{-1}cm^{-1}$ and expressed as enzyme unit $g^{-1}FW$.

Ascorbate peroxidase (EC 1.11.1.11) activity was measured according to Nakano and Asada[15] by estimating the rate of ascorbate oxidation. Reaction mixture (2ml) consisted of 25mM phosphate buffer (pH 7.0), 0.1mM EDTA, 0.25mM Sodium ascorbate, 1.0 mM H_2O_2 and 0.2 ml of enzyme extract. The enzyme activity was determined using an extinction coefficient of 2.8$mM^{-1}cm^{-1}$ by measuring the change in absorbance at 290nm for 1 min and expressed as enzyme unit $g^{-1}FW$.

Guaiacol Peroxidase (EC 1.11.1.7) was assayed following Hameda and Klein[16]. The reaction mixture (2ml) consisted of 25mM phosphate buffer (pH 7.0), 0.1mM EDTA, 0.05% guaiacol, 1.0 mM H_2O_2 and 0.2 ml of enzyme extract. The increase in absorbance due to oxidation of guaiacol was monitored at 470nm. The enzyme activity was measured using extinction coefficient of 26.6 $mM^{-1}cm^{-1}$ and expressed as enzyme unit $g^{-1}FW$.

Determination of ascorbic acid (AsA) and non-protein thiol (NPSH) content: Leaves of pea seedlings were homogenized in a solution containing 50mmol L^{-1} Tris-HCl and 10 ml L^{-1} Triton X-100 (pH 7.5) and centrifuged at 6,800*g* for 10 minutes. The supernatant was mixed with 10% Trichloroacetic acid (TCA) in 1:1(v/v) and again centrifuged at 6,800*g* for 10 minutes to remove protein. Estimation of AsA was performed following the method of Jacques-Silva et al.[17]. An aliquot of sample (300ul) was incubated at 37°C in a medium containing 100µL TCA 13.3%, 100µL DW and 75 µL DNPH solution. The DNPH solution comprised 2% DNPH, 0.23% thiourea and 0.27% $CuSO_4$ diluted in 49% H_2SO_4. After 3hr, 500µL of 65% H_2SO_4 was added and samples were read at 520 nm. AsA was calculated with the standard curve obtained from L (+) ascorbic acid.

NPSH concentration was determined with Ellman's reagent [18]. 400 µL aliquot of sample was added to a medium containing 550 µL of 1 mol L^{-1} Tris-HCl (pH 7.4). Add 0.05ml of 10 m mol L^{-1} 5, 5-dithio-bis (2-nitrobenzoic acid) (DTNB) and developed colour was read at 412nm. A standard curve was constructed using cystein to calculate the amount of NPSH in samples.

Lipid peroxidation: The lipid peroxidation in leaves was measured by determining the malondialdehyde content according to Heath and Packer19. The plant material (200mg) was homogenized in 5 ml of 0.1% w/v trichloroacetic acid and centrifuged at 10,000g for 10 min. 1 ml of supernatant was mixed with 4 ml of 0.5% thiobarbituric acid (made in 20% trichloroacetic acid). The mixture was then heated at 950 C for 30 min and after cooling it was again centrifuged. The absorbance of supernatant was measured at 532 nm and corrected by subtracting the non-specific absorbance at 600nm. The MDA concentration was calculated using the extinction coefficient of 155 Mm-1 and expressed as n mol g-1 FW.

RESULTS

Table 1: Effect of various concentration of al on germination and seedling growth of *pisum sativum* l. Variety arkil and rachana

Combi-nations	Seed Germination (%)		Germination Rate		Seedling Height (cm)		Absolute Growth Rate		Dry Weight (mg/plant)	
	Arkil	Rachana	Arkil	Rachana	Arkil	Rachana	Arkil	Rachana	Arkil	Rachana
C	63.83±1.80	66.51±2.30	10.21±0.28	10.64±0.37	13.66±0.92	17.16±1.42	0.52±0.011	0.48±0.023	0.68±0.004	0.35±0.001
T1	58.92±5.15	49.10±1.03^{b}	9.42±0.82	7.85±0.16^{b}	11.36±0.36^{a}	14.30±0.49	0.18±0.014^{b}	0.37±0.014^{b}	0.24±0.002^{b}	0.32±0.003^{b}
T2	57.14±4.63	44.64±0.51^{b}	9.14±0.74	7.14±0.08^{b}	10.50±0.40^{b}	12.86±0.75^{b}	0.12±0.017^{b,y}	0.46±0.017^{x}	0.20±0.002^{b,y}	0.32±0.001^{b}
T3	50.00±1.54^{a}	37.50±	8.00±	6.00±	9.06±	11.46±	0.07±	0.36±	0.16±	0.31±
T4	41.50±	2.57^{b,y,*}	0.24^{a}	0.41^{b,y,*}	0.53^{b,x}	0.14^{b}	0.001^{b,y,*}	0.018^{b,*}	0.004^{b,y,**}	0.001^{b,**}
	1.80^{b,x,*}	39.28±	6.64±	6.28±	7.06±	10.60±	0.02±	0.34±	0.13±	0.27±
		1.03^{b,y}	0.28^{b,y,*}	0.16^{b,y}	0.29^{b,y,**}	0.30^{b,x}	0.005$^{b,y,**,\#}$	0.017^{b,**}	0.003$^{b,y,**,\#\#}$	0.001$^{b,y,**,\#\#}$

Data are mean of three replicates ± SEM. ^{a} P<0.05, ^{b} P<0.01 versus C, ^{x} P<0.05, ^{y} P<0.01 versus T1, * P<0.05, ** P<0.01 versus T2, $^{\#}$ P<0.05, $^{\#\#}$ P<0.01 versus T3. C, control; T1, 0.2 g Al Kg^{-1} soil; T2, 0.4 g Al Kg^{-1} soil; T3, 0.6 g Al Kg^{-1} soil and T4, 0.8 g Al Kg^{-1} soil

Seed germination and seedling growth: Seed germination and seedling growth of *Pisum sativum* (var. Arkil and Rachana) as affected by the stress of Al toxicity was evaluated in terms of germination rate, seedling height, AGR and DW (Table 1). Seed germination was significantly inhibited in both Arkil and Rachana variety of pea. Maximum inhibition of 34.98% in seed germination was recorded in Arkil at T4 treatment while in Rachana, it was declined maximum at T3 treatment (43.61%) when compared with their controls. The inhibition of seedling height, AGR and DW in Arkil and Rachana were found to be in dose-response manner. The inhibition in seedling height was significantly ($p<0.05$) at lower doses in both varieties of pea however in highest concentration of Al (0.8 g Kg^{-1} soil) it was reduced very significantly ($p<0.01$) as compared to control. AGR of Arkil was found to be reduced significantly in all treatment and as compared to T4 treatment control group exhibited 26 times more AGR. However, control group of Rachana had only 1.41 times more AGR when compared with highest dose of Al. Rachana variety had shown a slight increase in AGR at T2 treatment as compared to T1 treatment. DW of Arkil had also shown to be decreased more prominently in all treatment as compared to Rachana. A maximum decline of 80.88% and 22.85% of DW was recorded at T4 treatment in Arkil and Rachana respectively. Thus Rachana was more Al tolerant than Arkil (Table 1). Al remarkably ($p<0.01$) reduced the total soluble sugar (TSS) in both varieties of pea. Maximum amount of TSS was recorded in control groups of both varieties. A maximum of 3.38 and 1.84 times decline in TSS was observed at T4 treatment in Arkil and Rachana respectively when compared with their controls (Fig 1).

Photosynthetic pigments and protein content: Al variously affected the pigments and protein content of both varieties of pea. Total chlorophyll and carotenoid of Arkil and Rachana were found to be maximum in their control. A decrease in pigment content of both varieties obvious with the increase in Al concentratin except at T3 treatment. Highest concentrations of Al adversely affected the amount of chlorophyll and carotenoids. Maximum reduction of 76.38% and 56.32% in total chlorophyll and carotenoid was recorded in Arkil variety. By contrast, Rachana variety had shown a maximum decrease of 17.30% and 17.53% in total chlophyll and carotenoid content at T4 treatment (Table 2). Results show that protein content was decreased in both varieties of pea when compared with their controls. In Arkil variety, protein was found to be significantly declined at highest concentration of Al treatment (25.51%). However, in Rachana variety, protein content was first decreased at lower concentrations of Al and then a slight increased was recorded at higher concentrations of Al (0.6 and 0.8 g Kg^{-1}soil) as compared to T2 treatment (Table 2).

Table 2: Effect of various concentration of al on pigments and protein contents in leaves of *pisum sativum* l. Variety arkil and rachana

Combi-nations	Chlorophyll a (mg/g FW)		Chlorophyll b (mg/g FW)		Total chlorophyll (mg/g FW)		Carotenoids (mg/g FW)		Protein (mg/g FW)	
	Arkil	Rachana	Arkil	Rachana	Arkil	Rachana	Arkil	Rachana	Arkil	Rachana
C	1.87±0.009	1.61±0.015	1.73±0.115	0.47±0.023	3.60±0.002	2.08±0.009	128.75±2.03	137.68±1.30	15.95±1.44	14.61±0.63
Tl	1.48±0.020^{b}	1.60±0.014	0.67±0.017^{b}	0.43±0.011	2.15±0.037^{b}	2.03±0.002	117.08±1.73^{b}	130.55±0.86^{b}	14.71±0.34	13.08±0.44
T2	1.58±0.032^{b,x}	1.34±0.017^{b,y}	0.64±0.003^{b}	0.37±0.011^{b,x}	2.22±0.029^{b}	1.71±0.029^{b,y}	103.32±1.20^{b,y}	122.98±0.99^{b,y}	14.18±0.49	11.45±0.19^{a}
T3	1.57±	1.44±	0.47±	0.40±	2.05±	1.85±	1.35±	120.59±	12.58±	11.75±
T4	0.009^{b,x}	0.008^{b,y,**}	0.001^{b,y,**}	0.002^{a}	0.007^{b,x,**}	0.011^{b,y,**}	0.04^{b,y,**}	1.02^{b,y}	0.53	0.94^{a}
	0.66±	1.35±	0.18±	0.37±	0.85±	1.72±	56.23±	113.54±	11.88±	11.73±
	0.006$^{b,y,**,\#\#}$	0.009$^{b,y,\#\#}$	0.004$^{b,y,**,\#\#}$	0.011^{b,x}	0.002$^{b,y,**,\#\#}$	0.020$^{b,y,\#\#}$	0.01$^{b,y,**,\#\#}$	0.67$^{b,y,**,\#\#}$	0.86^{a}	0.70^{a}

Data are mean of three replicates ± SEM. ^{a} $P<0.05$, ^{b} $P<0.01$ versus C, ^{x} $P<0.05$, ^{y} $P<0.01$ versus T1, * $P<0.05$, ** $P<0.01$ versus T2, $^{\#}$ $P<0.05$, $^{\#\#}$ $P<0.01$ versus T3.
C, control; T1, 0.2 g Al Kg^{-1} soil; T2, 0.4 g Al Kg^{-1} soil; T3, 0.6 g Al Kg^{-1} soil and T4, 0.8 g Al Kg^{-1} soil.

Activities of antioxidants and lipid peroxidation: Antioxidant enzymes activity of two Al treated varieties were significantly increased with the increase of Al concentration in soil when compared to their control ($p<0.01$). Maximum SOD activity was recorded in control group of both varieties. The activity of SOD was noticeably in Arkil as compared to Rachana under different concentration of A (Fig 2)l. SOD activity in Rachana was increased compared to control (16.21%) when Al concentration in soil reached 0.4 g Kg^{-1}soil. Further increase in Al concentration seemed to produce no marked difference in plant response. CAT activity of both varieties increased, reaching the highest level at the 0.8 g Kg^{-1} soil concentration except at T1 treatment where Arkil variety had shown a slight reduction in CAT activity compared to control. This increase was more significant ($p<0.01$) in Rachana variety than in Arkil, especially at high Al stress (0.8 g Kg^{-1} soil). APX and GPX activity of both varieties increased significantly ($p<0.01$) at high toxic Al levels (0.6 and 0.8 g Kg^{-1} soil). This activation was more pronounced in Rachana variety than in Arkil at different Al concentrations (Fig 3). Protective mechanisms against Al stress seems to be conferred by the activation of SOD, CAT, APX and GPX activity. The effect of Al stress on cell membrane integrity was measured by evaluating MDA content of plant tissues. Compared to control seedlings a significant change (a 15.5 times increase in Arkil and 61 times increase in Rachana variety) in MDA concentration was observed at highest dose of Al.

Concentrations of AsA and NPSH: Al treatment led to increased tissue AsA concentration in both varieties of pea. Both lower and higher concentration of Al resulted in a significant increase in AsA content in both varieties and at highest concentration it resulted in a maximum enhancement of 150 and 153.1% in Arkil and Rachana variety respectively compared to their control. Furthermore, NPSH concentration increased remarkably in both varieties of pea increasing Al supply, as can be deduced from Fig 4. As compared to Rachana, NPSH concentration was more pronounced in Arkil variety of pea.

DISCUSSION

Seed germination and seedling growth: Al had effected crop production either by contamination or occurring naturally. A large genotypic variation in physiology, plant growth and quantity in response to Al is recorded[20, 21]. All treatment used in the present study caused a decrease in seed germination of both varieties of pea, throSugh at different extent. Beside treatment, several other environmental factors such as oxygen concentration, moisture level and temperature are known to exhibit an influence on seed germination[22]. Many other mutagenic agents and heavy metal have been shown to inhibit seed germination[23]. The reduced germination after Al treatment has also been recorded by Faheed[24]. The results also represented that Al treatment significantly reduced seedling growth of both varieties of pea and more inhibition was observed in Arkil variety as compared with Rachana. Dry weight has also been found to reduce in greater extent in Arkil when compared with Rachana variety. Delima and Copeland[25] reported that highly toxic Al concentration inhibits the growth of germinating wheat seeds. Many research groups have suggested about the integration of Al with many cellular sites like cell wall, plasma membrane, DNA etc. and by which it interfere with plant growth [7,26,27.].Al affected cell elongation by induced effects on microtubules and actin filament of root apices and causing growth inhibition by stabilizing microtubules in central elongation zone [28]. In Arkil variety, a significant decrease in the sugar content was observed at all concentrations of Al. HowSever, in Rachana it was remarkably decline only at highest concentration. As the concentration of Al increases the sugar content decrease which may be due to reduction in water uptake and less absorption of CO_2 during photosynthesisS[29]. Tondon and Gupta[30] have also reported a reduction in sugar contents at deviated doses of heavy metals.

Figure 1: Effect of various concentration of Al on sugar content in leaves of *Pisum sativum* L. variety Arkil and Rachana. Data are mean of three replicates ± SEM. [a]*P*<0.05, [b] *P*<0.01 versus C, [x] *P*<0.05, [y] *P*<0.01 versus T1. C, control; T1, 0.2 g Al Kg^{-1} soil; T2, 0.4 g Al Kg^{-1} soil; T3, 0.6 g Al Kg^{-1} soil and T4, 0.8 g Al Kg^{-1} soil.

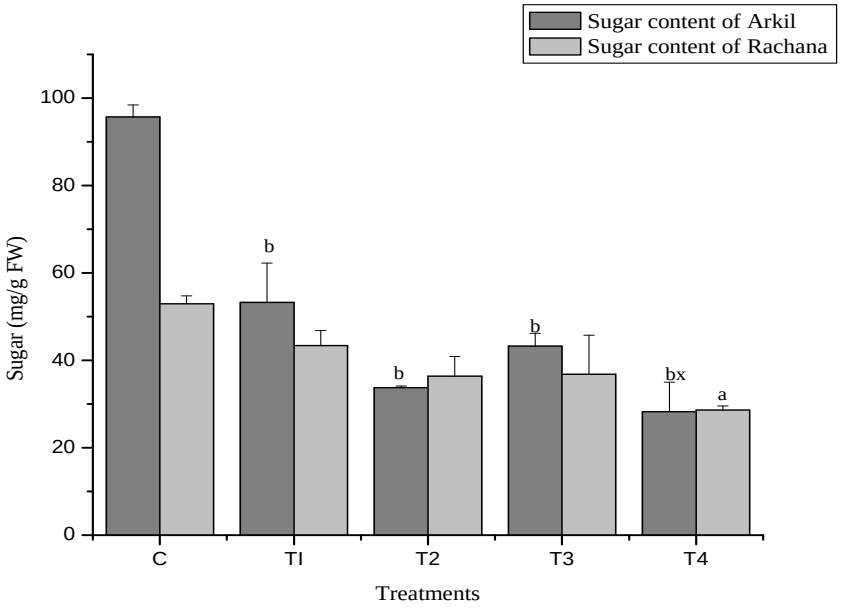


Figure 2: Effect of various concentration of Al on superoxide dismutase (SOD) and catalase (CAT) activity in leaves of Pisum sativum L. variety Arkil and Rachana. Data are mean of three replicates ± SEM. a P<0.05, bP<0.01 versus C, x P<0.05, y P<0.01 versus T1, * P<0.05, ** P<0.01 versus T2, # P<0.05, ## P<0.01 versus T3. C, control; T1, 0.2 g Al Kg-1 soil; T2, 0.4 g Al Kg-1 soil; T3, 0.6 g Al Kg-1 soil and T4, 0.8 g Al Kg-1 soil.

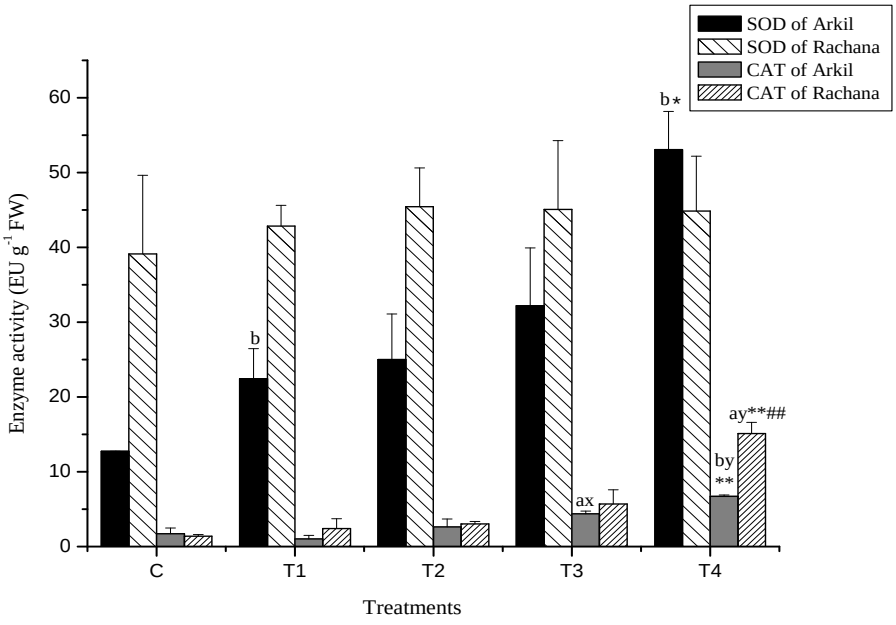


Figure 3: Effect of various concentration of Al on ascorbate (APX) and guicol peroxidase (GPX) activity in leaves of Pisum sativum L. variety Arkil and Rachana. Data are mean of three replicates ± SEM. a P<0.05, bP<0.01 versus C, x P<0.05, y P<0.01 versus T1, * P<0.05, ** P<0.01 versus T2, # P<0.05, ## P<0.01 versus T3. C, control; T1, 0.2 g Al Kg-1 soil; T2, 0.4 g Al Kg-1 soil; T3, 0.6 g Al Kg-1 soil and T4, 0.8 g Al Kg-1 soil.

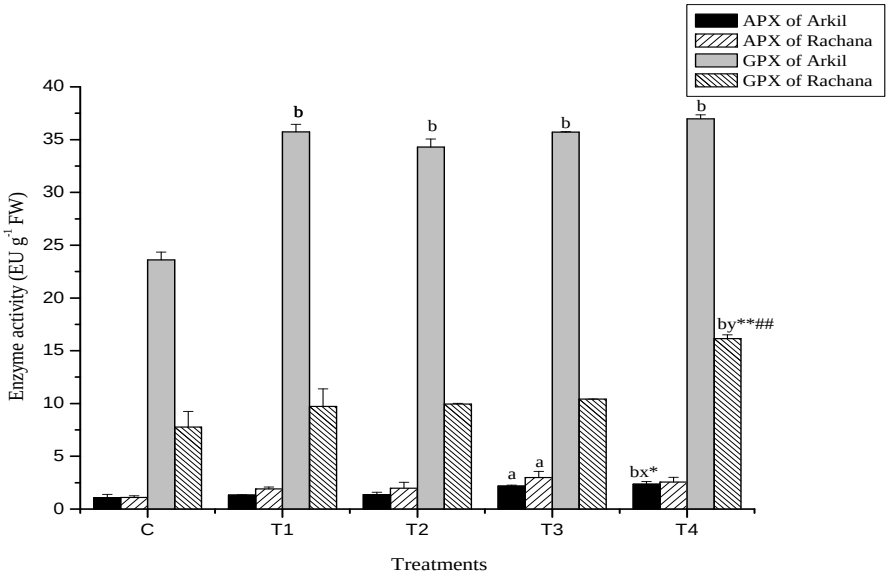


Figure 4: Effect of various concentration of Al on non protein thiol (NPSH) and ascorbic acid (ASA) content in leaves of Pisum sativum L. variety Arkil and Rachana. Data are mean of three replicates ± SEM. aP<0.05, bP<0.01 versus C, x P<0.05, y P<0.01 versus T1, * P<0.05, ** P<0.01 versus T2. C, control; T1, 0.2 g Al Kg-1 soil; T2, 0.4 g Al Kg-1 soil; T3, 0.6 g Al Kg-1 soil and T4, 0.8 g Al Kg-1 soil.

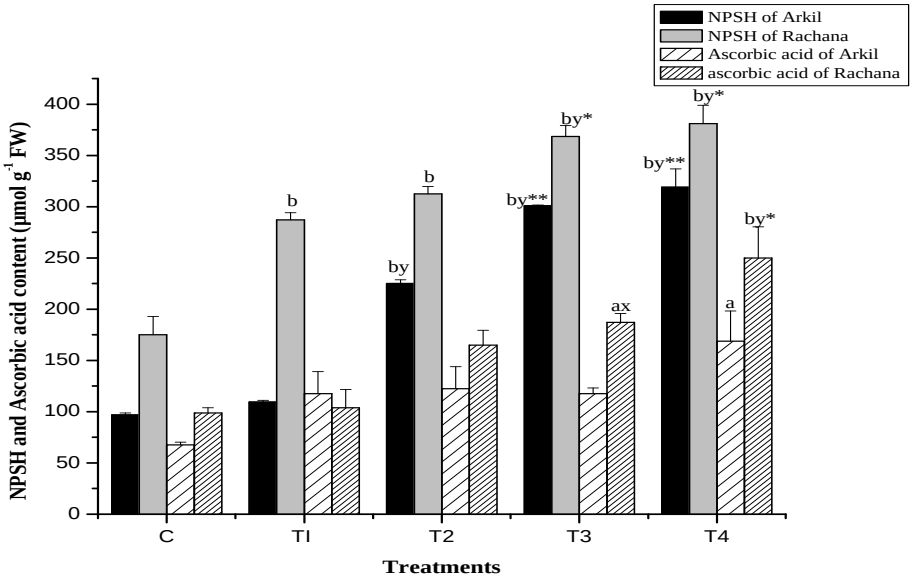
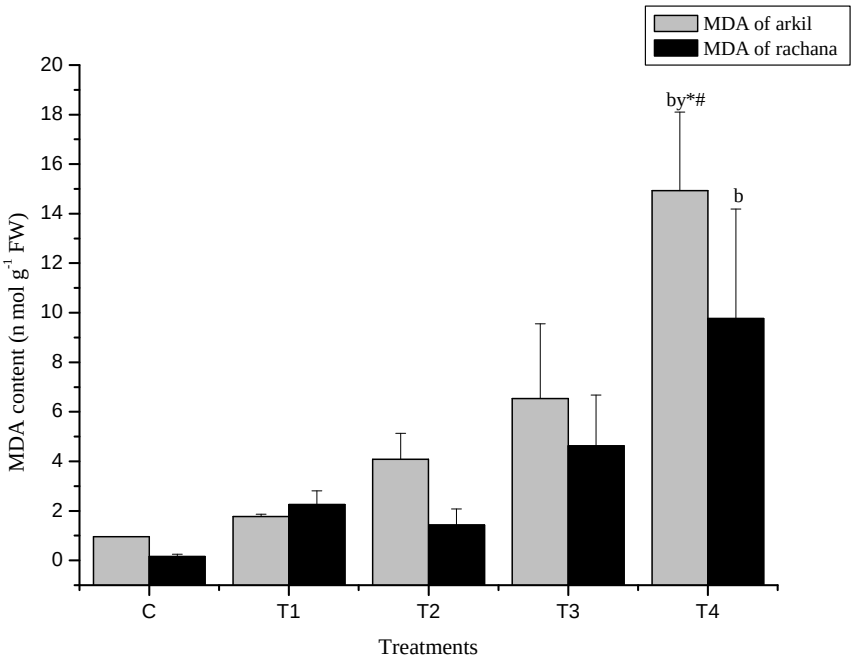


Figure 5: Effect of various concentration of Al on malondialdehyde (MDA) contents in leaves *Pisum sativum* L. variety Arkil and Rachana. Data are mean of three replicates ± SEM. [b] $P<0.01$ ver C, [y] $P<0.01$ versus T1, [*] $P<0.05$ versus T2, [#] $P<0.05$versus T3. C, control; T1, 0.2 g Al Kg^{-1} soil; T2, 0. Al Kg^{-1} soil; T3, 0.6 g Al Kg^{-1} soil and T4, 0.8 g Al Kg^{-1} soil.



Photosynthetic pigments and protein content: The results of the present study showed that a significant decrease in total chlorophyll and carotenoids content were observed in both varieties of pea at higher concentration of Al. Several investigations have shown that Al-induced reduction in Mg concentrations [31,32]. Therefore, the reduction observed in pigment content under Al stress may be due to decline in Mg concentrations and ultimately affected the photosynthetic capacity of pea plants. Carotenoids play an essential role in protecting the photosynthetic apparatus against stress and their loss usually brings about an increased production of excited singlet oxygen [33]. The results also showed that Al remarkably declines the protein content of pea seedling at higher doses in both varieties and more reduction was observed in Arkil as compared to Rachana variety. Samantary[34] have also reported earlier that metal induced inhibition of protein synthesis.

Antioxidant enzyme activity: Al toxicity enhanced production of ROS and plants main line of defence against oxidative stress to prevent formation of ROS [35,36]. In order to accomplish this task several ROS scavenging enzymes known as antioxidant are produced by the plants. The data presented in this study revealed that activities of SOD, CAT, APX and GPX were increased in both varieties of pea under Al stress. Metals disturb metabolic pathways, especially in the thylakoid membrane, which also results in increased formation of free radicals and reactive oxygen species. Some of Al induced genes are encoded for antioxidant enzyme (CAT, POX) thus suggesting a strong connection between Al stress and oxidative stress in plant [5,37]. Increased in ROS as result of the Al stress caused an enhancement in SOD activities and it may be dose-dependent [38]. Our results also indicate that the SOD activity

enhanced greatly in both varieties of pea under high Al stress. Superoxide dismutating $O_2^{\cdot-}$ to H_2O_2 and act as the first line of defense against ROS that produced under stress. The H_2O_2 so produced are subsequently detoxified by APX, GPX and CAT. There are atleast five different isoforms of APX present in thalakoid, stroma, cytosolic peroxisome and apoplast region of plant cell [39,40]. All these isoforms respond differentially to metabolic and environmental signals [41]. Result showed an increase in APX activity of pea plant under Al stress. H_2O_2 get detoxify and converted to water molecule through APX activity in both varieties of pea. Darko et al.[42] observed higher APX activity in Al-tolerant lines of wheat than Al-sensitive wheat lines. Our results demonstrated that GPX and CAT also involved in scavenging ROS in pea plant but differentially in both varieties.

Lipid peroxidation: Biochemical studies shows that Al ions have a strong binding capacity to biomembranes [43] which trigger the free radical chain reactions mediated by iron (Fe) ions and induce the peroxidation of lipids. Lipid peroxidation is the most evident symptom of oxidative stress. A dose-dependent increase in LP as observed in the present study agrees with some earlier studies conducted [6,44].

Concentrations of AsA and NPSH: To **understand** the contribution of non-enzymatic antioxidant against Al toxicity, we examined AsA and NPSH concentrations of pea seedling. AsA and NPSH concentration were enhanced under Al treatment compared to the control, indicating that AsA and NPSH are involved in antioxidant response of pea seedling to Al toxicity. It has been suggested that a high thiol pool is characteristic for metal toxicity [45]. Noctor[46] demonstrated that AsA is one of the most abundant antioxidants found in plant and it plays diverse physiological roles. AsA have the capacity to directly scavenging superoxide, hydroxyl radicals and singlet oxygen [47]. The elevation of AsA concentration together with the significant raise in NPSH levels may reflect a defense reaction to enhanced production of ROS [48] and indicate the capacity of pea seedling to tolerate the metal toxicity. From the present investigation, it is evident that Al phytotoxicity decreases seedling growth and induces oxidative stress in both varieties of pea. To combat this oxidative damage caused by Al, different antioxidants viz. SOD, CAT, APX, GPX, AsA and NPSH may serve as important role.

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