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Effect of Arsenic Exposure on *Triticum aestivum L.* Genotypes: Evaluation of Arsenic Accumulation in contrasting Genotype of India

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Abstract

During last few decades arsenic contamination has emerged as a major environment problem. The irrigation through arsenic-laden water significantly elevates the arsenic concentration in soil which influences the growth and development of different crops. It is necessary to identify wheat genotype that has ability to grow in arsenic contaminated soils with low arsenic uptake efficiency. A hydroponic method had proved useful tool for screening plants. The present study was conducted to identify contrasting As accumulating wheat genotypes by in different levels of arsenate 50µM, 250µM in hydroponic experiment. The results revealed that highest As uptake was observed in Pbw550 genotype (HAWG) and the lowest As uptake by Wh711 genotype (LAWG). All the fifteen wheat genotypes were ranked with respect to their As tolerance index: Wh711>Huw234>Pbw550>Pbw154>Dbw17>Wr544>Hd2733>Pbw502>Pbw343>Pbw2285> K307> Raj3765>Hd2329>Huw468>Rr21 respectively. The results suggested that (LAWG) was the most suitable wheat genotype for growth in As contaminated region.

Keywords: Wheat genotype, arsenic accumulation, tolerance index, translocation factor.

Introduction

Arsenic contamination is getting wider attention due to its adverse effect on human health and environment. Arsenic is recognized as one of the toxic and carcinogenic metalloid and it naturally occurs in soil at traces quantities[1,2]. It ranks No.1 of hazardous substance, due to its significant threat to human health and surrounding environment. Arsenic contaminated ground water is serious global environmental problem. High concentration of arsenic in ground water has been reported in many countries like Argentina, Bangladesh, China, India, Chile, Mexico, Nepal, Taiwan, Japan, Mongolia, Poland, Vietnam and USA[3-7] WHO recommended permissible limit of arsenic for drinking water ($0.01 mgl^{-1}$) and permissible limit recommended by FAO for irrigation water is ($0.10 mgl^{-1}$). The ground water in these countries has been extensively contaminated by arsenic and it gets accumulated in the soil through repeated irrigation. As a result arsenic uptake and accumulation by crops plants cause serious health problem in human beings and animals. Early seedling growth and germination of seeds are more susceptible to heavy metal stress as there is no resistance mechanism developed at this stage. Thus seed germination is widely used to assess phytotoxicity. Wheat ranks as the third most important cereal in terms of plantation area and production. So, far most of the research related to arsenic uptake by crops plant has been confined to rice plant[7-10], Wheat genotypes shows genetic variation in terms of uptake and metabolism in presence of arsenic which provides possible devise for agronomic practices to grow improved genotypes suited for arsenic contaminated soils. In arsenic affected lands farmers cannot manage to follow remediation of contaminated agriculture soil. So, to cope up with such threat by using low arsenic accumulating wheat genotypes provide an alternative for farmers to reduce its entry into human food chain.

Material and methods

Germination assay experiment: Wheat genotypes used in this experiment were certified and procured from National Seed Corporation of India. The concentrations of arsenate used were 50µM and 250µM as ($Na_2HAsO_4.7H_2O$) with control. All seeds were surface sterilized in 0.1% $HgCl_2$ solution for 30s, followed by thorough washing with MilliQ water and soaked in MilliQ for 24hr. Seeds were then transferred in sterilized petridish having Whatmann filter-paper moistened with 10mL of MilliQ water in controlled samples and arsenic solution in treated samples. Total 30 seeds of each genotype were taken and kept in the dark, at 25˚C for four days. After 4 days of incubation germinated seeds were counted when both the plumule and radical were extended. Germination percentage, germination index, relative germination rate and arsenic injury rates were measured in each treatment by the formula of Li (2008).

Hydroponic screening experiment: Experiment was done in the growth chamber under controlled environment. The day/night regime was 14/10h light/dark period (260-350mmol m-2.s-1) with an average temperature of 25˚C during day and 20˚C during the night and relative humidity was 50-70%. The germinated seedlings were taken and transferred into the plastic trays containing nutrient solution. Two levels of arsenic applied (50µM and 250 µM) with control each. Nutrient solution was replenished twice a week and pH of the solution was adjusted to 5.5 using 0.1 M KOH and HCl. All plants were harvested after

15 days of treatment application.

Quantification of arsenic concentration: In harvested plants root and shoot parts were separated and kept for drying at 65˚C in oven for two days. Dried plant material was mixed with nitric acid and perchloric acid (5:1 v/v) and digested in microwave digester (Speed wave digester BERGHOF speed wave Digester unit MWS-3+). The digested samples were filtered with Whatman-42 filter paper and diluted with Milli Q water and arsenic was measured by inductively coupled plasma mass spectrometry (ICP-MS) Agilent Technologies 7500 Series.

Quality control and quality assurance: The standard reference material of arsenic (consisting of 998±4 mgl^{-1}As-NIST and BAM-CRM traceable; EMerck, Germany) was used for each analytical batch. Analytical data quality was ensured with repeated analysis of quality control samples (n=3) and the results were within (±2.82 mgl^{-1}) limit of the certified values. Standard AA03N-3 (Accustandard, USA) was used as a matrix reference material which was spiked with known concentration (0-50 μgl^{-1}As) of standard reference material, and the recovery of total As was within 85.3% (±2.8; n=5) to 89.5%.

Statistical Analysis: Statistical analysis was performed by SPSS 16 software package by one-way ANOVA and Duncan multiple range test. Relative root- shoot length and arsenic concentration in root and shoot biomass were analysed by Duncan multiple range test (DMRT).

Results and Discussion

Different parameters were analyzed separately to obtain considerable data to process in order to make the comparison more reliable.

Germination assay of wheat genotype: Germination percentage, germination index, germination rate and extent of arsenic injury rate were examined. Significant differences (p<0.05) was observed in the mean values of germination percentage and germination index in two arsenic treatments (0, 50µM and 250µM). Seed germination was affected in 50µM As treatment while at 250µM As treatment germination was inhibited figure-1. The highest seed germination percentage was recorded in Pbw154 and Wh711 genotype (85.56% and 82.22%) at 50µM As treatment indicating high As tolerance figure-1. There were significant variations in seed germination percentage in As treatment in all the tested genotypes. At 250µM As treatment, Wh711 and Dbw17 genotypes had highest value of germination percentage (48.89% and 45.56%) and the lowest value was recorded in Pbw502 and Rr21 (25.56% and 26.67%) genotype in 250µM figure-1. The highest germination index (GI) value was recorded in Wh711 (6.17 and 3.67) in both arsenic (50 and 250µM) treatments and lowest (GI) value was recorded in Hd2733 and Pbw502 genotype (4.50 and 1.92) respectively table-1. There were declining trend in relative germination rate value with increasing As treatment and maximum reduction were occur in 250µM As treatment. Wh711, Pbw343 and Pbw154 genotypes exhibited significant difference (*P*<0.05) in relative germination rate in both As treatments. At high As treatment 250µM decrease in germination percentage was noticeable as As injury rate thus As induced injury was higher due to reduction in germination percentage. Highest As injury was recorded in Rr21 and the lowest As injury was recorded in Wh711, indicated better As tolerance of Wh711 genotype compared to other genotypes table-1.

Table-1
Effect of arsenate treatment on germination index, relative germination rate and As injury rate. One way ANOVA was applied and values are the average of three replicates with ±Standard deviation; *significant at 95% level

Wheat genotype	Germination index Control	Germination index 50 µM As	Germination index 250µM As	Relative Germination rate 50 µM As	Relative Germination rate 250 µM As	Arsenic injury 50 µM As	Arsenic injury 250 µM As
DBW-17	7.3±0.4	5.33±0.95	3.42±0.38	0.74±0.15	0.47±0.03	0.26±0.15	0.53±0.03
WH-711	7.5±0.0	6.17±0.38	3.67±0.63	0.82*±0.05	0.40*±0.04	0.18*±0.05	0.51*±0.08
K307	7.3±0.3	4.83±0.80	2.58±0.80	0.67±0.12	0.45±0.10	0.33±0.12	0.65±0.10
RR-21	7.4±0.1	5.33±0.88	2.00±0.66	0.74±0.12	0.53±0.12	0.72±0.13	0.73±0.09
PBW343	7.3±0.3	4.92±0.52	2.67±0.38	0.64*±0.12	0.42*±0.07	0.67±0.07	0.64±0.04
HUW468	7.5±0.0	5.08±0.63	3.33±0.72	0.70±0.09	0.48±0.10	0.68±0.08	0.56±0.10
PBW550	7.5±0.0	5.83±0.76	3.50±0.66	0.79±0.08	0.52±0.02	0.78±0.10	0.53±0.09
PBW154	7.3±0.3	6.42±0.29	2.67±0.76	0.92*±0.02	0.38*±0.12	0.89±0.05	0.63±0.09
HUW234	7.5±0.0	5.67±0.76	2.75±1.00	0.79±0.12	0.38±0.13	0.76±0.10	0.63±0.13
WR544	7.3±0.3	5.92±0.52	3.00±0.90	0.82±0.05	0.52±0.05	0.82±0.05	0.58±0.13
PBW502	7.5±0.0	5.25±1.00	1.92±0.52	0.75±0.13	0.43±0.09	0.70±0.13	0.74±0.07
HD-2733	7.5±0.0	4.50±0.50	3.08±1.18	0.65±0.07	0.45±0.18	0.60±0.07	0.59±0.16
HD-2324	7.4±0.1	6.08±0.38	3.17±0.95	0.82±0.07	0.43±0.12	0.82±0.07	0.57±0.12
HD-2285	7.3±0.3	4.67±0.76	2.42±0.63	0.68±0.13	0.37±0.11	0.64±0.08	0.67±0.09
Raj6735	7.3±0.1	5.00±1.00	2.92±0.76	0.70±0.12	0.43±0.13	0.68±0.15	0.60±0.11

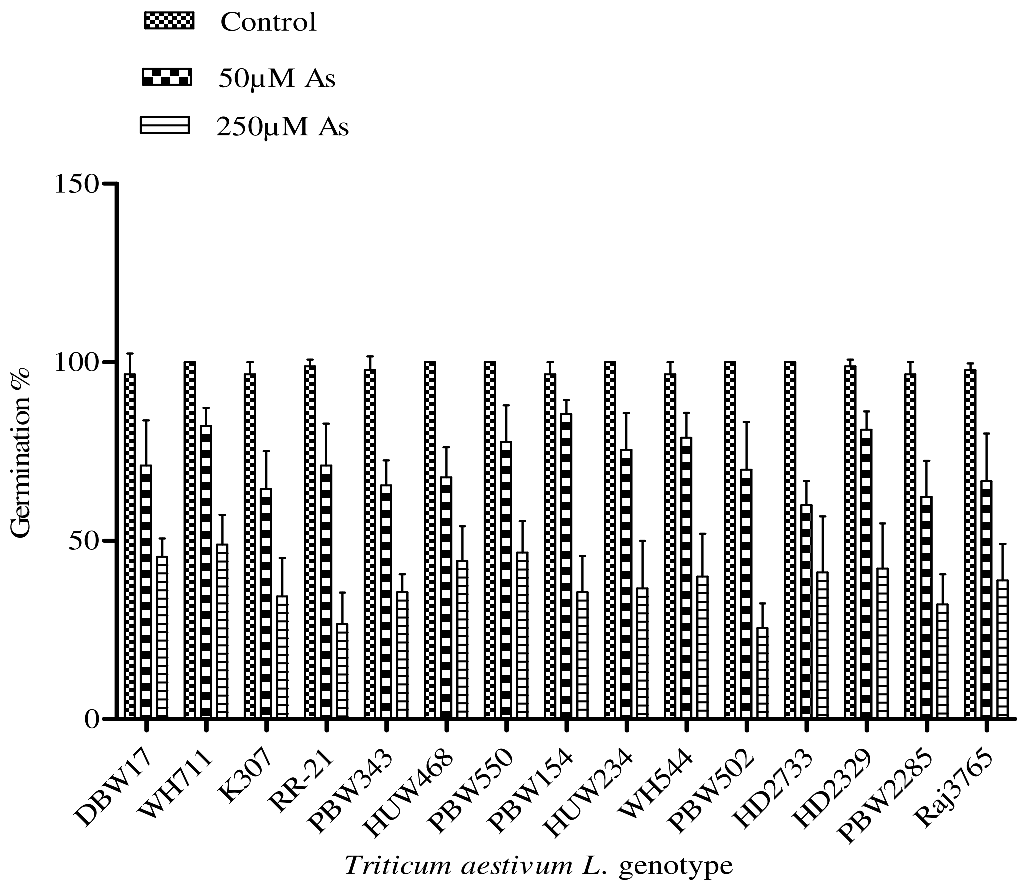

Figure-1
Effect of arsenate treatment on germination percent of fifteen wheat genotypes

Arsenic induced toxicity in wheat seedling: Screening experiment: High degrees of variation were seen in the growth parameters of wheat genotypes at higher arsenic concentration (250µM). Root and shoot length reduced prominently in presence of As in nutrient solution. According to results longest root length observed in K307 (8.70cm) genotype and the lowest was recorded in Wr544 (2.83cm) genotype at 50µM As treatment, but reduction occurs considerably at higher 250µM As treatment table-2. At 250µM As treatment Wh711 genotype had longest root length (8.37cm) and Hd2285 (2.67cm) genotype had lowest root length compared to other genotypes. In rest of the other genotypes effect of As on root length was in intermediate range. Shoot length markedly differed, Pbw343 genotype showed longest shoot length (23.60cm) and Pbw2285 showed the lowest shoot length (8.83cm) at 50µM of arsenic treatment. At 250µM of As Wh711 genotype recorded highest shoot length (23.60cm) value and Pbw2285 showed lowest (8.40cm) shoot length value table-2. There was reduction in the biomass of all genotypes in both the arsenic treatments table-3. Wh711genotype had highest biomass (25.40mg) at 250µM As treatment and the lowest biomass was recorded in Rr21 (13.50mg) genotype table-3.

Level of arsenic increased in all the genotypes with increase in As treatment. Root and shoot As value were increased significantly (P<0.05) table 2 in all the genotypes. The results revealed that in 50µM As treatment highest level of As in plant biomass was recorded in Pbw550 (9.10 µg/gm) and Wr544 genotype (8.60 µg/gm) and lowest level of As were recorded in K307 (0.92 µg/gm) and Rr21(1.17 µg/gm) genotype in dry weight figure-2. In 250µM As high value of arsenic in per plant biomass of Pbw550 genotype (42.4 µg/gm As/plant) and Hd2329 genotype (35.34 µg/gm As plant^{-1}) respectively. Lowest As value were recorded in Wh711 (3.85 µg/gm As plant^{-1}) and K307 genotypes (5.65 µg/gm As plant^{-1}) and rest other genotypes showed intermediate results figure-2 at 250µM As. Based on these results Pbw550 genotype categorized as high arsenic accumulating wheat genotype (HAWG) and Wh711 genotype categorized as low arsenic accumulating wheat genotype (LAWG).

Concentration and distribution of arsenic in wheat genotype:

Table-2
Effect of two arsenic treatments on root and shoot length of fifteen wheat genotype. Mean values in a column having same letter do not differ significantly at p≤0.05 level by Duncan's multiple range test

Wheat genotype	Root length Control	Root length 50µM As	Root length 250µM As	Shoot length Control	Shoot length 50µM As
DBW-17	9.17±0.85[a]	8.27±0.99[ab]	7.20±0.66d[b]	20.90±1.1[a]	19.43±1.8[a]
WH-711	9.20±0.92[a]	6.53±1.27[ab]	8.37±1.36[a]	19.23±1.2[a]	19.33±2.4[a]
K307	8.57±0.68[a]	8.70±0.90[a]	5.40±0.62[b]	19.73±1.6[a]	17.50±1.8[ab]
RR-21	10.30±0.62[a]	4.77±1.38[b]	4.23±0.85[b]	22.63±4.6[a]	17.40±1.7[ab]
PBW343	8.77±1.12[a]	5.40±0.62[a]	6.97±2.86[a]	20.53±3.9[a]	23.60±4.4[a]
HUW468	10.63±0.51[a]	8.13±1.14[b]	5.47±0.72[c]	26.30±1.0[a]	18.57±2.3[b]
PBW550	4.93±0.7[a]	4.40±0.7[a]	4.70±0.5[a]	19.63±3.9[a]	14.53±1.9[b]
PBW154	3.67±0.8[a]	3.23±1.9[a]	4.97±0.3[a]	15.07±1.4[a]	15.87±3.4[a]
HUW234	3.50±0.6[a]	3.63±0.6[a]	2.67±0.8[a]	14.37±1.7[a]	12.83±0.6[ab]
WR544	3.33±1.0[a]	2.83±0.7[a]	3.00±0.4[a]	13.07±2.2[a]	13.23±2.0[a]
PBW502	5.40±0.8[a]	5.57±3.0[a]	6.47±1.6[a]	12.27±0.9[a]	14.20±2.0[a]
HD-2733	4.60±0.4[a]	3.87±0.4[a]	4.00±0.7[a]	13.97±0.8[a]	14.37±0.9[a]
HD-2324	4.17±0.6[a]	3.83±0.8[a]	3.73±0.7[a]	15.87±1.7[a]	12.80±1.2[b]
PBW-2285	3.40±0.7[a]	3.20±0.5[a]	2.67±0.6[a]	9.80±0.3[a]	8.83±0.5[a]
Raj6735	4.67±0.8[a]	3.93±0.3[ab]	3.40±0.4[b]	16.73±1.2[a]	15.20±0.9[a]

Table-3
Effect of two arsenic treatments on biomass of fifteen wheat genotype. Mean values in a column having same letter do not differ significantly at p≤0.05 level by Duncan's multiple range test

Wheat genotype	Control	50µM As	250µM As
DBW-17	26.80±3.0[a]	19.80±1.1[b]	23.23±3.1[ab]
WH-711	25.13±4.0[a]	18.83±2.0[b]	25.40±2.7[a]
K307	20.93±2.4[b]	17.23±3.7[b]	16.60±1.9[a]
RR-21	32.27±4.1[b]	11.53±1.5[c]	13.50±1.0[a]
PBW343	23.87±4.7[a]	15.03±3.1[a]	18.57±5.0[a]
HUW468	26.60±3.2[a]	18.50±2.0[b]	15.37±2.5[b]
PBW550	24.83±3.0[a]	17.50±1.5[b]	22.03±1.2[a]
PBW154	21.13±0.9[a]	20.53±1.9[a]	18.37±1.4[a]
HUW234	23.77±3.0[a]	19.33±1.5[a]	23.03±3.0[a]
WR544	25.63±2.3[a]	17.23±3.9[b]	21.97±3.5[ab]
PBW502	23.77±2.8[a]	21.73±5.1[a]	19.33±1.8[a]
HD-2733	22.37±5.0[a]	19.07±1.6[a]	17.90±2.6[a]
HD-2324	28.20±5.1[a]	17.03±1.0[b]	17.83±1.5[b]
PBW-2285	22.63±2.4[a]	18.63±3.2[a]	17.90±1.2[a]
Raj6735	27.53±1.5[a]	21.53±1.2[b]	19.00±0.7[c]

Translocation factor and tolerance index: Translocation of arsenic significantly ($p<0.05$) varied statistically amongst all the tested genotypes in both the As treatments. Translocation factor is the measure of the metal translocation from the root to shoot part. Pbw550 genotype had highest TF (3.2 and 4.0) value and lowest was recorded in Wh711 genotype (0.5 and 0.2) figure-3 at 50µM and 250µM As treatments respectively. Hence Pbw 550 genotype translocates high level of arsenic from root to shoot part and Wh711 translocate low level of As from root to shoot. Therefore it has been concluded that considerable concentration of arsenic was accumulated by the roots of Pbw550 genotype and that was translocate into the shoot part. On the other hand, there was low As uptake by the roots of Wh711 genotype (LAWG) assumed less efficient to translocate As from root to shoot parts. Tolerance index (Ti) was calculated according to Wilkins [12]. (Ti) differed significantly in all the tested genotypes at 250µM As treatment high degree of variation in tolerance index was observed. Wh711 genotype showed the highest value of Ti while Rr21 genotype had lowest (Ti) value figure-4. All the genotypes were categorized according to decreasing value of tolerance index as follows: Wh711>Huw234> Pbw550> Pbw154> Dbw17> Wr544> Hd2722> Pbw502> Pbw343> Pbw225> K307> Raj3765> Hd2329> Huw468>Rr21 respectively figure-4. Seed germination assay is the most important parameter for studying the effect of heavy metals stress on seedling as seeds lack defence mechanism against stress[13]. Morphological response like root and shoot length, plant biomass are useful parameters to study different metal susceptibility in plants[14]. Decrease in root length in presence of arsenic has been reported by a number of workers in other plant also[15,16]. In present study germination percentage in Wh711 genotype at 250µM as treatment was found to be the highest among all the studied genotypes. The range of germination percentage was from (48.89% to 25.56%) in the tested genotypes at 250µM As treatment. The data suggested that germination parameters and root-shoot length and biomass decreased with increasing As concentration in nutrient solution. A decrease in root-shoot length of wheat genotypes with increasing arsenic treatment was earlier reported in other plant species[15-17]. The study demonstrated that genotypes exhibited variation in presence of arsenic. According to results range of As in dry biomass were from (0.92 to 9.10µg/g dw) in 50µM As treatment while in 250µM As treatment it range from (3.85 to 42.4µg/g dw) in all the studied wheat genotypes. The difference in response of each genotype can be further confirmed by the TF. In 250µM As treatment Pbw550 had high TF (4.0) value hence this genotype translocate As from root to shoot part compared to other genotypes. While Wh711 genotype recorded lowest TF value (0.2) in 250µM As treatment, suggested this genotype is less efficient to transfer As in biomass. (Ti) for all genotypes was evaluated to ascertain the finding of the results, its value ranged between 42.5 to 102.1%. Wh711 genotype had highest Ti value as compared to other genotypes at 250µM As treatment, hence it was least effected in presence of arsenic. All the studied genotypes explore in this study, Pbw550 accumulated relatively high level of As in biomass and Wh711 genotype accumulated low level of As. Estimation of germination assay parameters, TF and Ti parameters demonstrated that Pbw550 genotype is high arsenic accumulating wheat genotype (HAWG) and Wh711is low arsenic accumulating wheat genotype (LAWG).

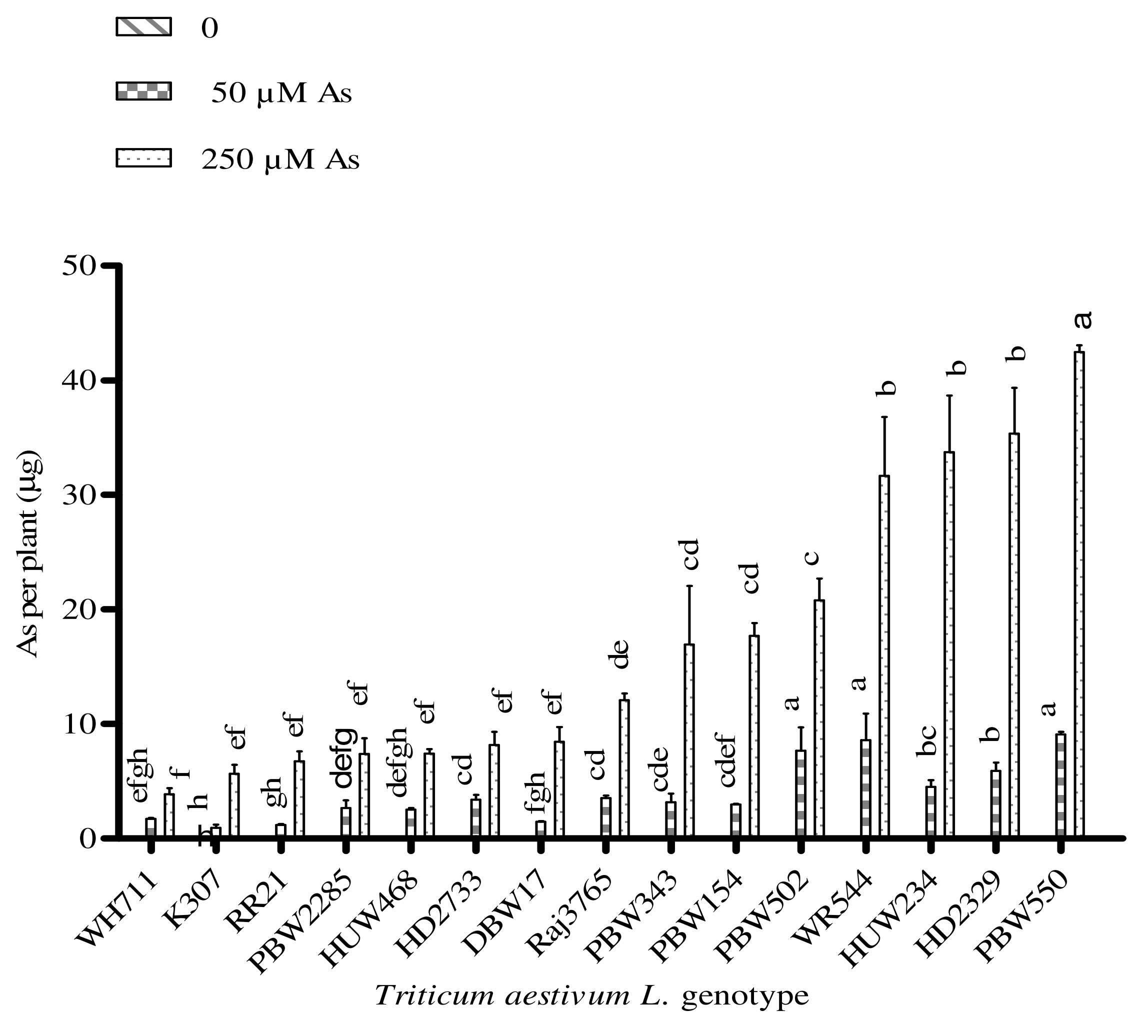

Figure-2
Shows arsenic concentration per plant to the applied two arsenic treatments 50 and 250µM

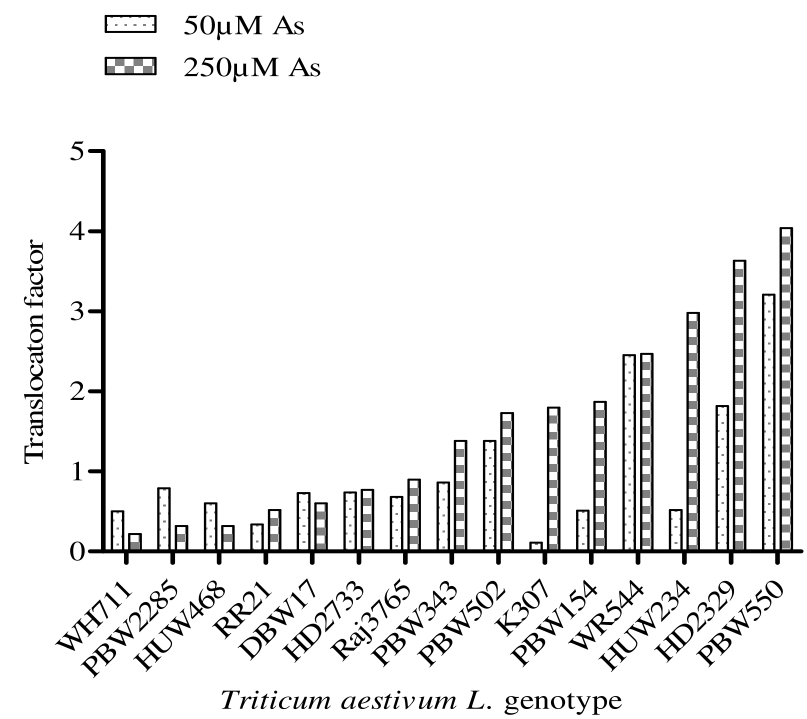

Figure-3
Shows translocation factor of fifteen wheat genotype to the applied two arsenic treatments 50and 250 µM

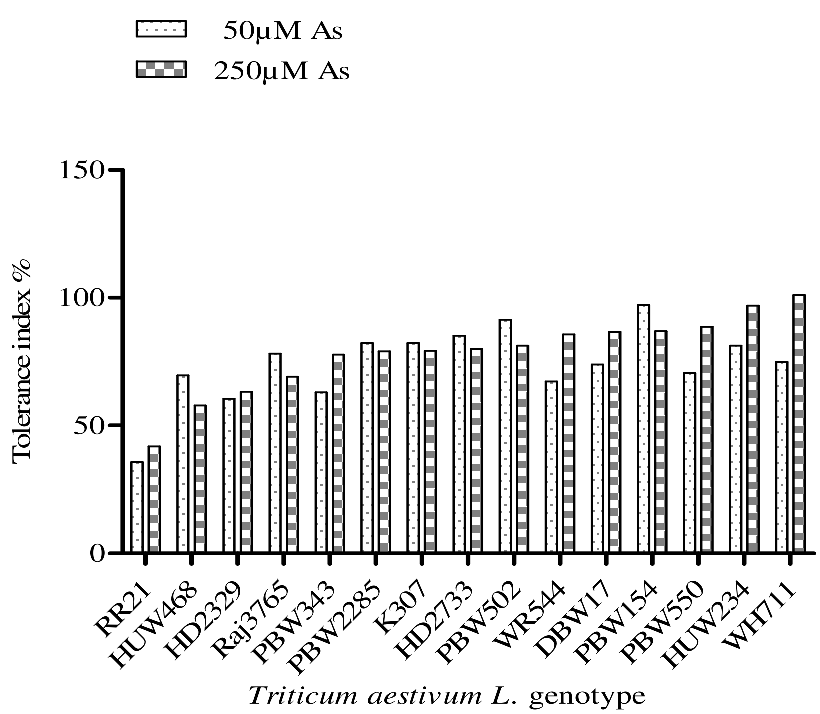

Figure-4
Tolerance index (Ti) of fifteen wheat genotype to the applied two arsenic treatments 50 and 250µ M

Conclusion

The screening results revealed that there was significant inhibition in germination, morphological and arsenic accumulation parameters in all the tested wheat genotypes. Fifteen commonly grown wheat genotypes in India evaluated, Pbw550 accumulates high level of arsenic categorized as high arsenic accumulating wheat genotype (HAWG). While Wh711 accumulated low level of arsenic categorized as low arsenic accumulating wheat genotype (LAWG). Hence (LAWG) genotype is the most appropriate for growth on arsenic contaminated region with economical produce. (LAWG) genotype had considerable potential to grow in the presence of high As concentration accompanied low arsenic uptake is fit to select for growth in As contaminated soil of India.

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