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RESEARCH ARTICLE

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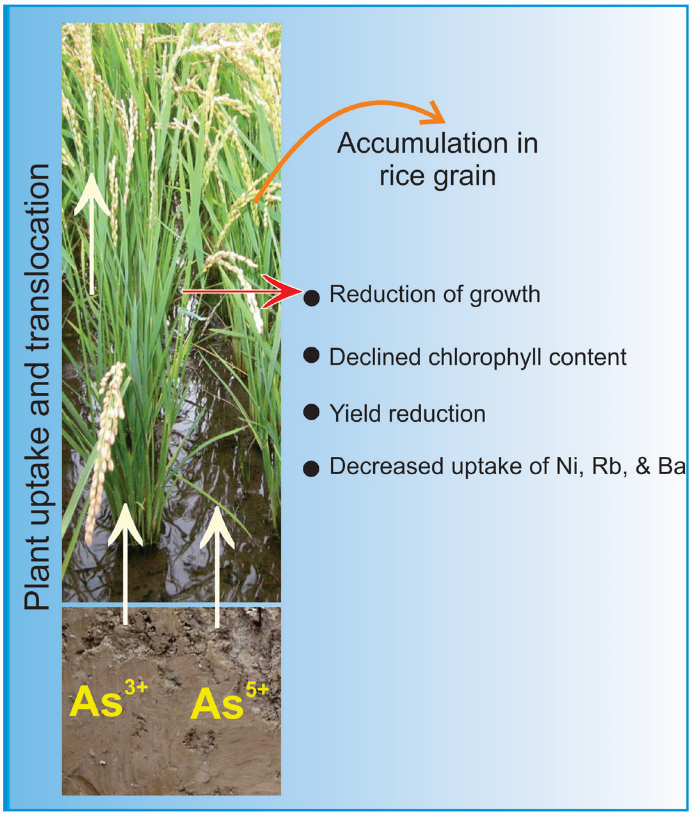

Highlights

- Case-control experiment was carried out to investigate the impact of arsenic (As) in soil on rice plants.
- Different rice varieties were cultivated in As-rich and low soils under controlled conditions.
- High As in soil affected the plant growth and yield.
- Some traditional genotypes showed tolerance to As stress.
- Such varieties can be used to develop improved rice types that can grow in high As soils.

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Growth responses of genetically diverse rice (*Oryza sativa* L.) cultivars to Arsenic stress

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Abstract: Although arsenic (As) is a toxic element for plant growth, it can accumulate in rice plants to higher levels. A case-control experiment was carried out to investigate the impact of As in soil on rice plant growth. Nine (9) different native and improved rice cultivars were grown in soils with 10 mg/kg As. Total As content in grains was quantified using an ICP-MS. The mean As content in control samples was 15.8 µg/kg while As treatment samples showed 122 µg/kg. Grains of improved rice variety of Bg 300 showed the highest As accumulation. The chlorophyll content declined by 28% under As stress and 56% tiller losses were also observed. The significant reduction of plant growth has given an impaired grain yield under As-treated conditions. Soil arsenic also showed a significant impact on Ni, Rb, and Ba uptake and accumulation. This study showed that As accumulation in rice is cultivar-dependent and also indicated a drastic reduction of the yield under elevated As levels. The native cultivar, *Kahawanu* was shown the lowest As-accumulation (90.1 mg/kg) under elevated As within the nine rice cultivars. So it can be identified as the best resistance cultivar for the As stress. The results can be utilized for developing high-tolerance rice varieties using new breeding technologies.

Keywords: arsenic stress; native rice cultivars; growth retardation; trace element accumulation;

INTRODUCTION

Arsenic is a naturally occurring metalloid, that occurred mainly in sulfide minerals, and weathering of such minerals leads to increasing arsenic in groundwater and soil (Zhao *et al*., 2010). Anthropogenic activities such as the use of arsenic-containing fertilizers, pesticides, herbicides, and organic manure could contribute to the elevated levels in soils, particularly in rice paddy soils. Triple superphosphate (TSP), which is highly used in rice cultivation, contains higher As content (Dissanayake & Chandrajith, 2009). Inorganic As is also identified as a class one non-threshold human chronic carcinogen (Meharg & Zhao, 2012). Chronic or acute exposure to high As leads to lung, bladder, liver, prostate, and skin cancers, leukaemia, diabetes, cardiovascular disorder, skin disorder, neurobehavioral abnormalities, miscarriage, and premature birth (Hong *et al.,* 2014, Gundert-Remy *et al.,* 2015, Hassan *et al.,* 2017, Watsoon *et al.,* 2022, Oberoi *et al.,* 2019)

Rice grain is well-known for its accumulation of As in excessive levels which is a few folds higher compared with other cereals (Williams *et al.,* 2007). Rice is a staple food, consumed throughout the world, particularly in Asian countries. However, it is one of the main sources of human exposure to inorganic arsenic (As), second after drinking water. Arsenic in rice plants increases under flooded conditions in paddy fields due to enhanced bioavailability that occurs with anaerobic conditions (Shah *et al.,* 2014). Particularly, As(V) is taken up into root cells through phosphate transporters due to their chemical analogy. Consequently, plant membrane transports are not able to discriminate between phosphate and inorganic As (Meharg & Zhao, 2012). As(III) uptake into root cells and then translocate into grains through Si-transport partway since similarity in their molecular size (Meharg & Zhao, 2012). Therefore, rice becomes one of the crucial sources of As in the human body.

In addition, higher As in rice plants disrupt cellular metabolism, impairs photosynthesis functions, alter the nutrient balance, and also cause toxicity by generating oxidative stress (Williams *et al.,* 2009, Lange *et al.,* 2020). Contents of chlorophyll-a and -b, which are the major photosynthetic pigments in plants showed a negative correlation with the As content in soils (Rahman *et al.,* 2007). The reduction of plant growth indicates the effect of As toxicity on rice plants which also reduces the net yield (Rahman *et al.,* 2007, Das *et al.,* 2013, Bakhat *et al.,* 2017). Higher As in rice plants lead to straight head disease, in which panicles remain upright due to the lack of grain filling and sterility (Kumarathilaka *et al.,* 2018). However, the uptake and accumulation of nutrients were greatly affected in rice-growing areas that were contaminated with As (Norton *et al.,* 2010, Williams *et al.,* 2009). The communities that consume largely rice-based foods suffer from major micronutrient deficiencies particularly iron (Fe) and zinc (Zn) while such deficiencies become more severe in As- contaminated rice growing areas (Dipti *et al.,* 2012, Duan *et al.,* 2013).

Sustainable production of rice can be achieved through paddy cultivation in soils with minimal levels of As or through growing rice cultivars that have a tolerance to high As levels (Kumarathilaka *et al.,* 2018, Meharg & Zhao, 2012, Rajatheja *et al.,* 2021). However, identifying As tolerance of diverse rice cultivars is important since it can be used as a genetic stock for breeding purposes. Therefore, this study was carried out to identify the genetic variability

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in As accumulation in rice grains using diverse rice cultivars and also investigated the effect of soil-As on plant growth and yield.

MATERIALS AND METHODS

A case-control plot experiment was carried out using soils treated with 10 mg/kg arsenic. A completely randomized block design was arranged with nine genetically diverse rice cultivars. Most commercially growing traditional rice varieties; *Suwadel*, *Beeheth heenati*, *Pokkali*, *Pachchapermumal*, *Madathawalu*, and *Kahawanu* and three improved rice varieties; At 362, Bg 300 and Bg 357 in which life spans are 3.0 to 3.5 months were selected for the plot experiment. Each pot was filled with 5 kg of natural soil in which the As level is known (0.60 mg/kg) and half of the pots were treated with 10 mg/kg As by adding sodium arsenate ($Na_2HAsO_4.7H_2O$). It was added 14 days before seed sowing as described by Smith *et al.* (2008). The crop establishment and management were carried out according to the recommendation of the Department of Agriculture, Sri Lanka.

Growth parameters, in terms of plant height, tiller count, and plant biomass and yield parameters, such as panicle number per plant, filled grains per panicle, panicle length, 100-grain weight, and total yield were determined at the harvesting stage. The total chlorophyll content was measured using the SPAD meter after 40 days from seed sowing. The harvested rice grains were oven-dried at 65 ^{o}C until achieving a constant weight. Dehusked grains were powdered using agate mortar and pestle. Approximately 0.10 g of the sample was digested with 2 mL of H_2O_2 (35 wt. %; Sigma Aldrich, Germany) and 5 mL of Conc. HNO_3 (69 % Traceselect; Fluka, Switzerland) in CEM-Mars 6 microwave digester, equipped with EasyPrep-Plus high-pressure vessels. Samples were digested at 180 ^{o}C for 10 minutes with a ramp time of 20 minutes. Solutions were then analyzed for As and other trace elements content using a high-resolution inductively coupled plasma mass spectrometer (Thermo ICapQ). Instrument calibration was performed using multi-element standards (Sigma-Aldrich, Germany). ^{75}Re and ^{103}Rh were used as internal calibration standards. A method blank was included in each digestion batch of 10, to verify any contamination and 10% duplicate samples were added. Certified reference material (CRM) provided by the National Institute of Environmental Studies (NIES) Japan No. 10d unpolished rice flour was also included to validate the analytical procedure. Recoveries of CRM were within ±1% for Mn and ±10% for Cu, Zn, and Cd, while recoveries were slightly higher for K and Fe.

The capability of translocation from soil to grains was determined by calculating the Accumulation Factor (AF) (Tang et al., 2021). The AF value for As was calculated as;

$$AF = \frac{\text{Grain As content}}{\text{soil As content}}$$

Two-way ANOVA, post-hoc test (Duncan test), and Pearson's correlation coefficient (r) were calculated using IBM SPSS 16.0 statistical software (version 22). Significant effects of independent variables were determined at $p < 0.05$.

RESULTS

Arsenic content in rice grains was eight-fold higher under As-treated conditions (mean=122 µg/kg) compared to that of the control plots (mean=15.8 µg/kg) that contained only As from natural soil (0.60 mg/kg). A factorial ANOVA test was performed to identify the effects of soil As and rice variety as well as their influence on grain accumulation. The level of As in the soil had a significant impact on grain As accumulation ($p < 0.01$), and different varieties also showed a significant impact on grain As accumulation ($p < 0.01$). The interaction effect was significant ($p < 0.01$), indicating that there was a combined effect of soil As level and varieties on As accumulation. Arsenic content in different rice varieties varied in the order Bg 300 > *Pachchperumal* > Bg 357 > *Beheth heenati* > *Madathawalu* > At 362 > *Suwadel* > *Pokkali* > *Kahawanu,* indicating the probable influence of genetic factors on the accumulation of As in rice that was grown under the elevated As conditions (Figure 1a).

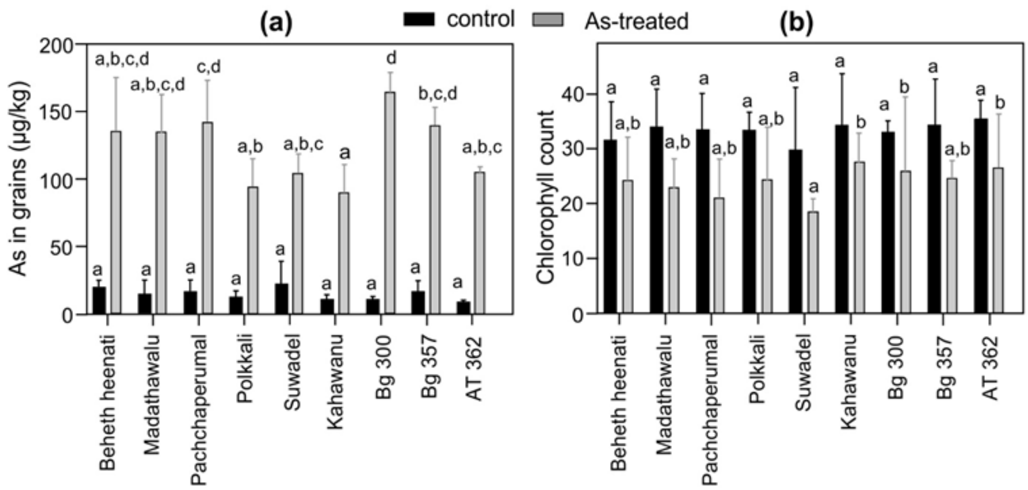

Figure 1: Variation of (a) mean grain arsenic levels (µg/kg) and (b) mean chlorophyll content (SPAD value) in genetically diverse rice cultivars under two different soil As levels. Columns not connected by the same letter are significantly different.

The pot experiment also showed that the accumulation of As in red rice was slightly higher (123 ±7.8 µg/kg) compared to that of in white rice varieties (121±9.7 µg/kg) that grow under As treated plots. When compared with the improved varieties (133±9.5 µg/kg), traditional rice varieties (119±7.6 µg/kg) showed lower As levels in grains under As-treated conditions. However, higher As accumulation was recorded in traditional varieties (16.6±2.2 µg/kg) than in improved varieties (13.7±2.0 µg/kg) grown under control conditions (Table 1). The capability of translocation of As from soil to grains decreased with the elevated As level. The mean AF value was 0.029 in control plots (range: 0.010 – 0.058) while it was 0.012 in As treated samples (range: 0.007 - 0.023).

Table 1: Summary statistics of grain arsenic content (µg/kg) under two different soil As levels (Mean ± SE).

	Control	As treated
All (n=27)	15.8 ± 1.7	122 ± 6.0
Newly Improved (n=9)	13.7 ± 2.0	133 ± 9.5
Traditional (n=18)	16.6 ± 2.2	119 ± 7.6
Brown rice (n=15)	15.4 ± 1.8	123 ± 7.8
White rice (n=12)	16.3 ± 3.0	121 ± 9.7

It was observed that higher As uptake led to the yellowing of plant leaves as noted after 40 days of seed sowing. The total chlorophyll content of rice leaf indicated that higher soil As concentrations significantly affected the reduction of the chlorophyll production ($p < 0.05$) in which a 28% reduction of chlorophyll content was observed under As stress (Table 2). The chlorophyll content (SPAD unit) in improved varieties (25.5±1.2) was comparatively higher than that of traditional varieties (23.1±0.8) in As-treated pots. Since the chlorophyll content directly affects photosynthesis, a comparatively higher impact can be expected on traditional varieties (29%) when compared to improved varieties (25%) (Table 2).

The addition of 10 mg/kg As to the soil significantly and adversely affected the plant growth parameters such as plant height, total tillers count and shoot biomass production, and also the yield parameters (Figure 2; Table 3). Moreover, genetically diverse rice cultivars provided a higher diversity in both growth and yield parameters. Approximately, a 56% reduction in tiller production was observed in As treated plots (8±0.6 tillers) than in non-treated rice plants (19±1.6 tillers). Arsenic toxicity in tiller production affected the improved rice cultivars (63%) more than the traditional rice cultivars (52%). The total biomass decreased by 36% under elevated As conditions (15.7±0.7 g) than in control plots (24.7±1.1 g). In traditional rice cultivars, 38% of biomass reduction was observed compared to improved varieties (33%) under high As conditions. The significant variation in plant height between control and As treated pots were observed only in the vegetative stage ($p<0.05$). In the latter period, plants recovered and, the variation became non-significant ($p>0.05$). In yield parameters, Panicle length, 100-grain weight, and filled grain number per panicle also declined under As treated plots and showed significant variation.

Trace element accumulation in rice gains

Among trace elements, manganese (Mn), copper (Cu), zinc (Zn), iron (Fe), nickel (Ni), cobalt (Co), and molybdenum (Mo) are required for the metabolism and maintaining a physiological balance of the human body. Such elements also act as cofactors in enzymatic reactions but can be toxic when exposed to higher concentrations (Dissanayake & Chandrajith, 1999). Moreover, these elements are also considered essential micro-nutrients for rice plants which directly and indirectly affected plant growth and development, but they are only required in small quantities (Das, 2014). The essential trace elements' accumulation in rice grains was investigated under both controlled and As treated soils which are shown in Table 4.

The concentration of Zn in rice grains ranged from 16.8 to 46.2 mg/kg in control pots while 11.2 to 35.5 mg/kg in As-treated pots. Zn accumulation in traditional varieties (26.6±1.3 mg/kg) was comparatively higher than that in improved varieties (18.5±1.6 mg/kg) under elevated As in soils. Moreover, white rice (24.8±2.2 mg/kg) showed a comparatively higher Zn concentration in rice grains than red rice (23.2±1.5 mg/kg). Iron concentration in rice grains varied from 7.2 to 60.9 mg/kg in control pots while 12.1 to 52.9 mg/kg in As treated pots. Improved rice varieties (26.6±4.8 mg/kg) showed lower Fe accumulation compared

Table 2: Summary statistics of chlorophyll content and plant growth parameters under two different soil arsenic levels at the vegetative stage (mean ± SE).

		Chlorophyll content	Tiller count	Plant height (cm)
All varieties	Control	33.1 ±0.6	3 ± 0.16	66.5 ± 3.1
	As 10+	23.9 ± 0.7	2 ± 0.15	50.5 ± 1.8
	% decrease	28%	48%	24%
Traditional varieties	Control	32.6 ± 0.8	3 ± 0.24	70.8 ±3.9
	As 10+	23.1 ± 0.8	2 ± 0.20	53.0 ± 2.3
	% decrease	29%	45%	25%
Improved varieties	Control	34.2 ± 0.7	3 ± 0.15	57.8 ± 3.5
	As 10+	25.5 ± 1.2	2 ± 0.24	45.5 ± 2.5
	% decrease	25%	50%	21%

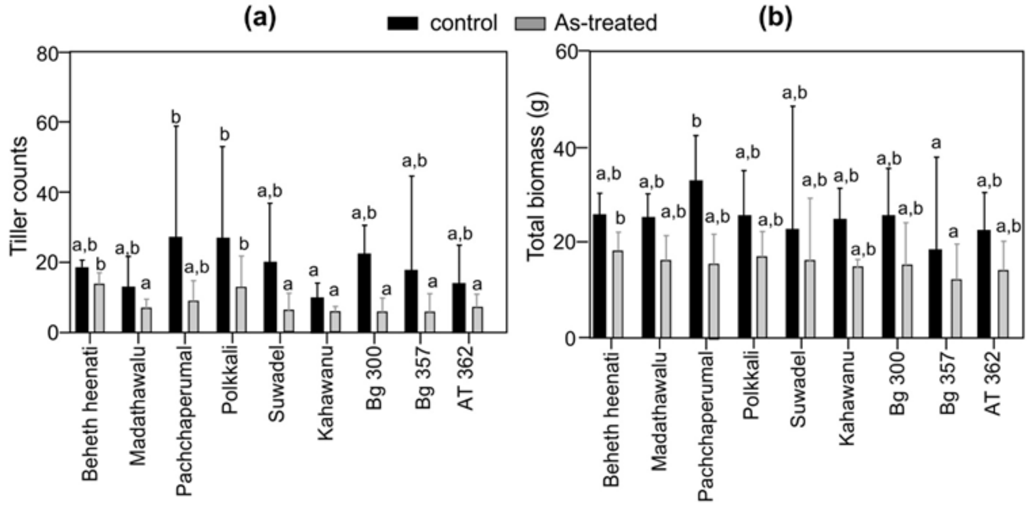

Figure 2: Variation of (a) tiller count and (b) total biomass (g) in genetically diverse rice cultivars under two different soil As levels. Columns not connected by the same letter are significantly different.

Table 3: Summary statistics of plant growth and yield parameters under two different soil As levels at harvesting stages (mean ± SE).

		Tiller count	Plant height (cm)	Total biomass (g)	Filled grain/ Panicle	Panicle length (cm)	100-grain weight (g)
All	Control	19 ± 1.6	110 ± 4.2	24.7 ± 1.1	192 ± 6.1	21.6 ± 0.38	1.79 ± 0.08
	As +	8 ± 0.6	107 ± 4.1	15.7 ± 0.7	181 ± 5.6	20.8 ± 0.37	1.76 ± 0.08
	% decrease	56%	3%	36%	6%	4%	2%
Traditional	Control	19 ± 2.2	121 ± 4.1	26.0 ± 1.4	193 ± 8.1	22.2 ± 0.5	1.64 ± 0.10
	As+	9 ± 0.9	117 ± 4.3	16.0 ± 0.6	183 ± 7.9	21.3 ± 0.5	1.62 ± 0.10
	% decrease	52%	4%	38%	5%	4%	1%
Improved	Control	18 ± 2.4	88.9 ± 3.7	22.2 ± 1.9	192 ± 9.0	20.6 ± 0.4	2.1 ± 0.05
	As+	7 ± 0.5	87.2 ± 3.8	14.9 ± 1.7	176 ± 6.2	19.8 ± 0.3	2.1 ± 0.06
	% decrease	63%	2%	33%	8%	4%	2%

to traditional cultivars (27.8±2.7 mg/kg) under elevated As.

The soil As level significantly affected Ni accumulation ($p < 0.01$). Ni is another essential element, but its deficiency is less common in humans. Ni concentration in rice grains ranged from 0.27 to 2.49 mg/kg in control plots and from 0.06 to 1.55 mg/kg in As treated plots. The accumulation of Ni in traditional varieties (0.77±0.13 mg/kg) was higher compared to improved varieties (0.43±1.0 mg/kg) under elevated As conditions. Furthermore, red rice showed higher Ni (1.05±0.14 mg/kg) than white rice (0.77±0.08 mg/kg) in control plots, but no remarkable differences were observed under As treated plots.

Other essential trace elements; Mo accumulation was reduced by 8% under the elevated As a condition, while Mn contents were increased by 6%. Cu and Co contents in grains were not observed any difference between As added condition and controls. The mean content of Mn, Cu, and Mo under elevated conditions were 18.5, 6.4, and 0.91 mg/kg, respectively while the mean content of Co was 52.8 µg/kg. The soil As level did not show any significant effect on these elements' accumulation in rice grains (p > 0.05).

Cadmium is a well-known carcinogen for humans and can seriously damage kidneys, lungs, and bones under higher intake (Chunhabundit, 2016). It is also considered a toxic element to plants. The Cd concentration in rice grains ranged from 6.8 to 72.2 µg/kg in control pots and from 4.7 to 70.0 µg/kg in As-treated pots. In improved varieties under elevated As in soils, Cd accumulation was comparatively higher (31.3 ± 6.7 µg/kg) than that of traditional cultivars (26 ± 2.8 µg/kg). Furthermore, red rice (30.5 ± 4.3 µg/kg) showed higher Cd content than that of white rice (23.3 ±2.4 µg/kg) in As-treated pots. However, Cd accumulation only decreased by about 2% under elevated soil As which did not show a significant impact on Cd accumulation (p > 0.05). But, a significant negative correlation was observed between grain Cd and As under elevated As soil conditions.

Aluminium (Al), chromium (Cr), vanadium (V), lithium (Li), rubidium (Rb), strontium (Sr), and barium (Ba) are considered potentially toxic elements to humans but may have some beneficial effects under ultra-low concentrations. The mean content of Al, Cr, Rb, Ba, and Sr were 16.5, 1.61, 0.72, 1.39, and 0.66 mg/kg in As-treated pots, respectively while the mean content of V and Li were 70.9 and 27.7 µg/kg, respectively (Table 5). It was noted that the accumulation of Al, Cr, V, Rb, Ba, and Sr in rice grains was decreased under As stress by 23, 11, 12, 34, 37, and 13%, respectively while Li concentration was increased by 9%. The soil As level significantly affected only the Rb and Ba accumulations ($p < 0.01$).

DISCUSSION

Arsenic accumulation in rice grains increases significantly with elevated As in soil (Das *et al.,* 2013, Williams *et al.,* 2009). Arsenic accumulation in rice grains was eight-fold higher under 10 $mgAskg^{-1}$ plots compared to that of the control plots in the study. However, the soil-grain transfer factor (AF) decreased with the elevated As level. In higher soil As concentrations, plant growth can be inhibited and cellular functions can be impaired (Suriyagoda *et al.,* 2018). Lipid peroxidation and other tissue damage by reactive oxygen stress, nutritional dynamics, and physiological stress induced by As exposure may affect the reduction of the AF value (Williams *et al.,* 2007).

Improved variety, Bg 300 showed significantly higher As accumulation that grew under As treated plots while the lowest As accumulating rice cultivar was *Kahawanu* followed by *Pokkali* and At 362. Rice cultivars with high radial oxygen loss and root porosity can diffuse more O_2 into the rhizosphere. It helps to oxidize highly mobile As(III) into less mobile As(V) and also enhances Fe(III) plaque formation (Kumarathilaka *et al.,* 2018). Some rice plants show a high rate of As detoxification mechanisms and alter the expression of transporters associated with the As metabolism more than other cultivars (Kumarathilaka *et al.,* 2018). The mutated genes that are related to Si or P uptake decrease the As uptake by the plant (Hu *et al.,* 2018, Limmer *et al.,* 2018). It may reflect the difference in As accumulation in rice varieties. Rice cultivars with more tolerance tend to accumulate a bigger amount of arsenic compounds (He *et al.,* 2021). More than that, under field conditions, variable results can be obtained due to the variations in environmental conditions, and field management activities (Ahmed *et al.,* 2011, Norton *et al.,* 2010, Rajatheja *et al.,* 2021). Soil Property may be the key factor controlling As availability in soil (Romero-Freire *et al.,* 2015).

When compared with the traditional varieties, improved rice varieties showed higher As levels in grains under As-treated conditions and lower As content under control conditions. Diyabalanage *et al.* (2016) observed higher arsenic content in traditional rice grown in rice fields of Sri Lanka compared to improved varieties, however, Edirisinghe & Jinadasa (2020) noted lower As contents in traditional varieties grown in rice fields in the North Central province. As uptake and accumulation may not depend on variety type; traditional and improved, only variety (genotype) may affect As accumulation.

The variation of leaf chlorophyll content of different rice varieties in the vegetative stage correlated with the As accumulating in grains. *Kahawanu* was found as the lowest As-accumulating traditional variety and the same variety was noted as the highest chlorophyll content that has grown under As-treated conditions (Figure 1b). The reduction of the chlorophyll content at the panicle initiation stage and the flowering stage was also reported under high As stress (Das *et al.,* 2013, Rahman *et al.,* 2007). Arsenic toxicity is caused for altering the chloroplast shape, shortening the longitudinal axis in the plant cell, concaving the membrane, and impairing the formation of grana. It directly affected decreased photosynthesis (Das *et al.,* 2013, Rahman *et al.,* 2007).

Rice plants in As-treated pots showed a longer vegetative period with a delayed flowering time of about 7 to 14 days compared to non-treated plots in all rice cultivars. The prolonged vegetative growth of rice plants could extend exposure to As species in the soil (Pillai *et al.,* 2010). The largest As-accumulating cultivar, Bg 300 showed the highest decline in tiller production (73%) under As stress while the second largest As-accumulating cultivar, *Pachchperumal* were shown the highest decline in biomass production (52%) under elevated As. Both inorganic arsenic forms, As (III) and As (V) are toxic to cellular metabolism. Elevated As level in the soil increases the As uptake in rice plants alters the photosynthesis function, damages the cell membrane, inhibits ATP synthesis, disrupts rice carbohydrates metabolism, and diminishes nutrient uptake. The germination percentage, the shoot and root elongation, water uptake, transpiration rate and absorptive functions were impaired adversely due to As toxicity which directly affected the plant growth. (Suriyagoda *et al.,* 2018, Williams *et al.,* 2009). Das *et al.* (2013) noted a severe effect (81% yield reduction) under 60 mg As/Kg.

Trace elements accumulation in rice grains

Both Zn and Fe play a vital role in the human body, showing deficiencies in the areas where people consume rice as their prime dietary source (Dipti *et al.,* 2012). It was observed that the Zn accumulation was comparatively lower under high As in soil in which an overall 7% reduction was noted. In a previous study, a 20% decrease in Zn accumulation was observed under 10 mg/kg As in soil (Williams *et al.,* 2009). Iron plaque build-up by oxidizing ferrous iron and forming iron oxides/hydroxides on the root surface and act as a barrier to uptake As into root cells, since Fe-oxides and oxyhydroxides are strong sorbents for As in the soil solution (Hussain *et al.,* 2021). Nearly a 14% decrease in Fe accumulation was observed under high soil As content in this study. However, soil As level did not show a significant impact on the Fe accumulation in rice grains. Norton et al. (2010) observed positive and negative relationships in Fe and Zn with As accumulation in grains and concluded that such conflicting correlations might have been due to variations in the field conditions other than genetic effects.

Excess accumulation of Cd occurs under aerobic conditions of paddy fields as it can increase bioavailability. On the other hand, aerobic soil conditions during the rice growth were effective in decreasing As uptake by the rice plant (Hu *et al.,* 2018). It may be the reason for the significant negative correlation between grain Cd and As under elevated As soil conditions. Accumulation of Ni in all investigated varieties except *Suwadel* showed decreasing trend under As treated conditions, an overall reduction of 34% was observed which showed a significant impact on Ni accumulation. In a previous study, mean grain Ni content decreased by 40% under similar treatment conditions (Williams *et al.,*

Table 4: Summary Statistics of essential elements' accumulation in rice grains (mg/kg) in two different soil As levels (Accumulated amounts of Co and V are represented by µg/kg, other elements' accumulation are represented by mg/kg).

	Mn*	Mn**	Fe*	Fe**	Cu*	Cu**	Zn*	Zn**	Mo*	Mo**	V*	V**	Ni*	Ni**	Co*	Co**
All																
Min	8.6	9.7	7.2	12.1	4.8	3.8	16.8	11.2	0.41	0.28	22.8	25.0	0.27	0.06	15.3	17.8
Max	28.2	30.5	60.9	52.9	9.9	11.2	46.2	35.5	1.95	1.77	212	198	2.49	1.55	147	116.9
Mean ± SE	17.5±1.0	18.5±1.1	31.8±2.8	27.4±2.3	6.4±0.2	6.4±0.3	25.6±1.2	23.8±1.2	1.0±0.1.0	0.91±00.1	80.3±80.2	70.9±60.8	0.94±00.1	0.63±0.1.0	52.9±5.0	52.8±4.2
SD	5.6	6.1	14.2	12.2	1.2	1.8	6.3	6.7	0.37	0.32	44.9	37.5	0.56	0.42	27.3	23.1
Improved																
Min	8.6	12.1	7.2	12.5	4.8	3.8	16.8	11.2	0.89	0.80	22.8	31.9	0.37	0.12	15.3	28.9
Max	25.9	25.8	37.2	52.5	9.9	8.5	32.0	27.1	1.34	1.77	186	100.4	1.29	1.02	92.8	56.4
Mean ± SE	14.7±1.7	19.0±1.7	21.5±3.5	26.6±4.8	6.5±0.5	5.7±0.5	23.1±1.7	18.5±1.6	1.05±00.04	1.17±00.1	66.5±16.2	65.2±80.5	0.66±00.09	0.43±1.0	43.1±8.5	43.8±3.6
SD	5.2	5.0	9.9	14.5	1.5	1.5	5.2	4.9	0.13	0.34	48.7	25.6	0.28	0.3	25.5	10.9
Traditional																
Min	8.8	9.7	17.5	12.1	4.9	4.2	18.4	13.3	0.41	0.28	38.5	25.0	0.27	0.19	27.2	17.8
Max	28.2	30.5	60.9	52.9	8.4	11.2	46.2	35.5	1.95	1.15	212.2	197.5	2.49	1.55	108.7	116.9
Mean ± SE	18.7±1.2	18.2±1.4	36.3±3.2	27.8±2.7	6.4±0.2	6.7±0.4	26.7±1.4	26.1±1.3	0.97±00.1	0.8±0.0.5	86.1±70.5	73.3±11.7	1.07±00.1	0.77±0.13	52.6±5.0	56.7±7.3
SD	5.5	6.6	13.6	11.3	1.1	1.9	6.6	6.1	0.43	0.23	43.0	41.9	0.61		18.9	26.0
Red rice																
Min	8.6	9.7	7.2	12.1	4.8	4.2	16.8	13.3	0.41	0.28	22.8	25.0	0.27	0.06	15.3	17.8
Max	28.2	30.5	60.9	52.9	8.4	11.2	46.2	34.0	1.95	1.15	212	145.1	2.49	1.52	147	97.1
Mean ± SE	16.5±1.2	15.9±1.3	33.9±4.2	26.4±2.8	6.3±0.3	6.6±0.5	25.1±1.7	23.2±1.5	1.0±0.1.0	0.82±00.1	83.4±10.9	62.4±70.4	1.05±00.14	0.60±0.1.0	50.2±7.5	46.2±5.1
SD	5.2	5.6	16.4	11.6	1.1	2.0	7.0	6.2	0.47	0.23	46.1	31.4	0.67	0.44	31.6	21.7
White rice																
Min	9.1	12.1	15.7	12.5	4.9	3.8	18.5	11.2	0.69	0.58	33.6	44.1	0.44	0.19	25.2	35.1
Max	27.9	27.3	51.3	52.5	9.9	8.8	38.6	35.5	1.16	1.77	186.8	198	1.29	1.55	92.8	116.9
Mean ± SE	19.2±1.9	22.2±1.4	28.8±3.2	29.2±4.3	6.6±0.4	6.1±0.5	26.4±1.5	24.8±2.2	0.99±00.04	1.05±00.01	75.6±12.9	83.6±12.5	0.77±00.08	0.67±0.12.0	56.9±5.7	62.8±6.5
SD	6.3	4.8	10.5	13.6	1.4	1.7	5.4	7.6	0.13	0.37	44.6	43.4	0.27	0.40	19.8	22.3

* - Control, **- As treated (10 mg/kg As)

Table 5: Summary Statistics of potentially toxic elements in rice grains in two different soil As treatment (Cd and Li are represented by µg/kg, other elements are represented by mg/kg)

	Li*	Li**	Al*	Al**	Cr*	Cr**	Rb*	Rb**	Sr*	Sr**	Cd*	Cd**	Ba*	Ba**
All														
Min	4.5	5.7	8.7	5.1	0.16	0.12	0.55	0.49	0.43	0.28	6.8	4.7	0.88	0.47
Max	60.5	45.6	63.7	33.6	6.96	4.46	2.48	1.14	1.60	1.32	72.2	70.0	4.47	3.30
Mean ± SE	25.4±2.8	21.4±2.4	27.7±2.7	16.5±1.5	1.81±0.4	1.61±0.23	1.09±0.09	0.72±0.03	0.76±0.05	0.66±0.04	28.1±3.5	27.6±2.8	2.18±0.2	1.39±0.13
SD	15.0	12.5	14.8	8.0	1.93	1.17	0.46	0.19	0.30	0.24	19.0	15.3	0.88	0.71
Improved														
Min	8.7	5.7	8.7	8.0	0.16	0.4	0.55	0.60	0.43	0.42	6.8	8.1	0.88	0.47
Max	43.4	21.6	50.8	29.7	6.05	3.8	2.07	1.14	0.78	0.76	68.8	70.0	3.79	1.95
Mean± SE	28.1±4.5	13.9±2.3	24.6±4.3	15.2±2.5	1.46±0.6	1.7±0.5	1.07±0.14	0.79±0.08	0.59±0.05	0.59±0.04	35.40±8.0	31.3±6.7	1.93±0.3	1.12±0.14
SD	12.7	6.5	12.9	7.4	1.90	1.3	0.43	0.23	0.14	0.13	24.1	20.2	0.95	0.43
Traditional														
Min	4.5	9.2	9.2	5.1	0.41	0.12	0.70	0.49	0.50	0.28	7.9	4.7	1.07	0.5
Max	60.5	45.6	63.7	33.6	6.96	4.46	2.48	1.12	1.60	1.32	72.2	60.5	4.47	3.30
Mean± SE	24.4±3.6	24.4±2.9	29.0±3.4	17.1±.1.9	1.99±0.5	1.59±0.3	1.1±0.1	0.69±0.04	0.84±0.1	0.70±0.06	25.0±3.5	26.0±2.8	2.29±0.2	1.5±0.18
SD	16.0	13.2	15.6	8.3	1.97	1.15	0.49	0.17	0.32	0.27	16.0	13.0	0.85	0.79
Red rice														
Min	5.8	5.7	9.2	5.1	0.16	0.12	0.55	0.49	0.44	0.28	7.9	4.7	0.88	0.47
Max	50.3	45.6	63.7	33.6	6.96	4.46	2.07	0.98	1.21	1.32	72.2	70.0	2.70	2.95
Mean± SE	25.1±3.2	21.8±3.2	26.9±3.6	15.8±1.9	2.1±0.56	1.58±0.3	0.95±0.09	0.68±0.03	0.72±0.06	0.65±0.06	31.7±5.3	30.5±4.3	1.94±0.14	1.28±0.17
SD	13.6	13.4	15.4	7.7	2.16	1.23	0.37	0.14	0.24	0.26	22.6	18.2	0.58	0.70
White rice														
Min	4.5	10.4	8.7	8.0	0.41	0.48	0.84	0.49	0.43	0.42	6.8	8.1	1.09	0.72
Max	60.5	42.7	50.8	31.7	6.05	3.75	2.48	1.14	1.60	1.05	42.1	39.7	4.47	3.3
Mean± SE	25.9±5.7	20.6±3.6	28.8±4.1	17.4±2.6	1.45±0.5	1.65 ±0.4	1.27±0.15	0.79±0.07	0.82±0.11	0.69±0.06	22.6±2.9	23.3±2.4	2.55±0.33	1.53±0.21
SD	18.0	11.4	14.4	8.6	1.61	1.15	0.53	0.24	0.37	0.22	10.1	8.3	1.14	0.74

* - Control, **- As treated

2009). Norton *et al.* (2010) observed a significant negative correlation between grain As and Ni accumulations in a field-based experiment.

The soil As level significantly affected the Rb and Ba accumulations ($p < 0.01$). This may be due to the possible interaction among Rb, Cu, and As in which metal-arsenide can form. For instance, $Rb_3Cu_3As_2$ (trirubidium tri-copper diarsenide) is a solid crystal formed in soil when As is high (Ovchinnikov *et al.,* 2018). This interaction may be the reason for reducing Rb accumulation in rice grains. Ba is favoured to bind with As and formed $BaHAsO_4.H_2O$ under low and neutral soil pH (3.63-7.43) (Zhu *et al.,* 2005) and this interaction may be the reason behind reducing Ba uptake into rice grains.

CONCLUSIONS

The results of this study provided information on the drastic reduction of plant growth and yield; especially tiller production under high As in soils. Traditional rice cultivar, *Kahawanu* can be recommended as a better cultivar to grow in As-contaminated soil considering its resistance under elevated As. Soil arsenic showed a significant effect on Ni, Rb, and Ba uptake and accumulation. Moreover, the results can be used for developing low arsenic accumulating but, high-yielding improved rice varieties through marker-assisted breeding or molecular biology.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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