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Interactive effects of arsenic and chromium stresses on mineral and metal uptake in jute (*Corchorus olitorius* L.)

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Abstract

Arsenic (As) and Chromium (Cr) toxicity often occurs concurrently in agricultural soils, which lead to a significant decline in crop growth and yield. A pot experiment was conducted to investigate the effects of As and Cr in the soil on the uptake of mineral elements as well as As and Cr in the two jute varieties differing in Cr tolerance during May to October, 2013. Here we report the effects of combined As and Cr stresses on plant biomass, photosynthesis, metal and nutrient uptake compared to As or Cr stress alone. Chromium tolerant variety, O-795, had significantly ($p \leq 0.05$) higher Cr and As levels in roots, stems and leaf tissues than Cr sensitive variety, O-9897. Roots had much higher As and Cr contents than above-ground parts. Arsenic stress reduced potassium (K^+), magnesium (Mg^{2+}), iron (Fe^{3+}), copper (Cu^{2+}), manganese (Mn^{2+}) and zinc (Zn^{2+}) contents in the roots and inhibited calcium (Ca^{2+}), and from being translocated into shoots and leaves. Chromium stress resulted in decreased concentration of K^+, Mg^{2+}, Fe^{3+}, Zn^{2+}, Cu^{2+} and Mn^{2+} and increased concentrations of Ca^{2+} concentration in the root tissues. Furthermore, translocation of all nutrients from roots to upper parts of plants was inhibited except Ca^{2+}. The combined stresses of low level of As (50 mg kg^{-1}) plus each Cr treatment showed less inhibition of nutrient uptake, in both varieties when compared with each Cr stress alone, indicating that low levels As plus Cr showed beneficial effect than single stress. In contrast, high levels (100 mg kg^{-1}) of As plus Cr showed further decrease in all nutrient concentrations except Ca^{2+} in all plant parts. These results suggest that the combined toxicity effect of low level As plus Cr on jute plant was lower than that of Cr or As treatment alone. Moreover, the reduction was more pronounced significantly ($p \leq 0.05$) in Cr sensitive variety O-9897.

Keywords: arsenic, chromium, mineral nutrient, variety, Jute (*Corchorus olitorius* L.).

Abbreviations: Cr_ Chromium; As_ arsenic; Pn_Net photosynthetic rate; Gs_Stomatal conductance; E_Transpiration rate

Introduction

Chromium (Cr) is one of the most abundant heavy metals on earth and a major contributor of ground water, soil and sediment contaminations. Contamination of agricultural fields with Cr is very toxic to plant and human health when it enters the food chain. This has been a major environmental concern over the last few decades (Tiwari et al., 2013). The release of Cr-compounds to the environment is mainly from electroplating, leather tanning, metal finishing, corrosion control and pigment manufacturing industries (Liu et al., 2011). Chromium has two stable interconvertible forms, trivalent Cr (III) and hexavalent Cr (VI) form, and between these two forms, the latter one is more toxic. High amounts of Cr in the soil can reduce plant growth and yield. However, some plants are able to withstand very high levels of Cr by adjusting their physiological mechanisms. Photosynthesis, water relations and mineral nutrition are commonly reported physiological processes that are affected by the Cr stress (Shanker et al., 2005). Moreover, influence of Cr on nutrient uptake has been reported for rice (Zeng et al., 2010), soybean (Moral et al., 1995), and tomato (Khan et al., 2000). Arsenic (As) is another toxic metal that occurs naturally. As is carcinogenic and its toxicity has become a global concern owing to the increasing contamination of water, soil and crops in many regions of the world (Rahman et al., 2007). In plant, As interferes with metabolism and inhibits growth by affecting nutrient uptake and distribution as well as by competing directly with nutrients and/or altering metabolic processes (Meharg and Hartley-Whitaker, 2002; Tu and Ma, 2005). For instance, Carbonell-Barrachina et al. (1997) noted reductions in boron (B), copper (Cu^{2+}), manganese (Mn^{2+}), zinc (Zn^{2+}), potassium (K^+), calcium (Ca^{2+}) and magnesium (Mg^{2+}) uptake in tomato plants.

As and Cr toxicity can arise concomitantly in soil due to a number of factors. Firstly, both Cr and As are present in the Earth's crust, ranking 7 and 1, respectively among the top 20 hazardous substances (ATSDR, 2005). They are released into the environment through anthropogenic sources including mining, agricultural activities and petroleum refineries (Martinez-Sanchez et al., 2011; Kabata-Pendias, 2011) and reached to toxic levels (Sridhar et al., 2011). Secondly, industrial pollutants also have considerable amounts of As and Cr (Sun et al., 2008; Zeng et al., 2010). In addition, extensive applications of organic (such as biosolids) and inorganic fertilizers containing As and Cr can increase appreciable the amount of both elements in soil (Oliveira et al., 2014; Zarcinas et al., 1996). Moreover, both Cr and As are released from wood preservative like chromated-copper-arsenate (CCA) to the environment. CCA readily leaches from wood, which results in the extensive spreading of soil contamination by As and Cr (Hingston et al., 2001; Kumpiene et al., 2008). Therefore, there is an urgent need to study the interactive effects of As and Cr on crop growth and mineral nutrition. Numerous studies have investigated the toxicity of Cr or As individually on plant growth and physiology. In spite of its, coexistence in the environment, information on plant response to the combined effects of Cr and As are scarce. Assessment of their combined toxicity is more relevant because it mimics actual polluted field even though their complexity involved in combined metal uptake by plants. Interaction of heavy metals can also affect the uptake and accumulation of these heavy metals and mineral nutrients in plants. For instance, Cr and As in *Pteris vittata* L. (Oliveira et al., 2014), As and Cd in rice seedling (Sun et al., 2008), Al and Cr in barley (Ali et al., 2011), salinity and Cr in barley (Ali et al., 2012), Cu and Cd in rice (Huang et al., 2009) have been documented. However, a combined effect on crop plants has never been studied. In this study, we have screened these two jute varieties in our earlier report (Islam et al., 2014) as tolerant variety (O-795) and sensitive variety (O-9897). We used two jute varieties to explore the As and Cr interaction on nutrient and metal uptake. Tossa jute (*Corchorus olitorius* L.) is a fiber producing crop ranked second in the world after cotton, in terms of global production, consumption and availability (Ranjit et al., 2013). It is an annual crop plant with tall stem and deep penetrating tap root. The plant grows fast even in nutrient-poor soils and produces a large amounts of valuable biomass. The lingo-cellulosic fiber is completely biodegradable, recyclable and eco-friendly. So far, most studies are focused on response of plants to single stress but in nature plants often face multiple stresses, the interaction of which may be far from additive. However, to our best knowledge, interaction of As and Cr stresses on nutrient uptake in crops has not been reported yet. Thus, it is of significant importance to determine the combined effects of As and Cr stress on uptake of the mineral nutrition. Here we report the combined effects of As and Cr stresses on uptake and accumulation of As, Cr and mineral nutrients in two jute varieties.

Results

Plant dry biomass

The dry weights of the two jute varieties, O-795 and O-9897, are shown in Fig. 1. Exposure of jute varieties, O-795 and O-9897 to As and Cr stresses resulted in a significant decrease in dry biomass of roots, stems and leafs except O-795 variety at 100 mg kg^{-1} Cr where there was no significant difference between Cr and control treatment. However, O-9897 showed more reductions than O-795 for each organ dry biomass in all treatments relative to control. Combination of low level As (50 mg kg^{-1}) and Cr stimulated dry biomass and alleviated Cr stress, but at higher level As (100 mg kg^{-1}) plus Cr caused further reduction of these dry biomass as compared to the As or Cr stress alone. Moreover, the variety O-9897 had a greater reduction than variety O-795, in every stress (Fig. 1) suggesting that the variety O-795 was more tolerant under Cr plus As stresses than the variety O-9897.

Gas exchange

The effects of As and Cr treatments on photosynthetic rate (Pn), Stomatal conductance (Gs) and transpiration rate (E) of two jute varieties are presented in Table 1. For the both varieties, addition of As significantly decreased Pn, Gs and E compared with control (Table 1). Cr exposed to soil, at low level (100 mg kg^{-1}) had no significant effect on Pn, Gs and E value, but at high levels (200 and 400 mg kg^{-1}) a significant reduction of all gas exchange parameters were found O-795 variety. On the other hand Cr sensitive variety, O-9897, had significantly lower Pn, Gs and E value compared to control at all Cr level. There by the effect of Cr treatment on Pn, Gs and E value of jute varieties under stress varied with crop variety and Cr level. Combined toxicity of the As (50 mg kg^{-1}) plus Cr treatment resulted in a slight increase in gas exchange parameters but at a high level of As (100 mg kg^{-1}) plus Cr decreased Pn, Gs and E significantly compared to As and Cr treatment alone which indicate that low level of As plus Cr had a beneficial effect on both varieties. Overall, for all stress treatments, O-795 showed higher Pn, Gs and E values than O-9897.

Cr and As accumulation

Cr and As concentrations in roots, stems and leaves of two jute varieties *viz.* O-795 and O-9897 are presented in Fig. 2 and 3. The Cr concentration in root, stem and leaf was increased dramatically with increasing Cr concentration, being significantly higher in roots than in stems and leaves (Fig. 2). Addition of As in the soil resulted in a significant increase of As concentration in all plant parts (Fig. 3). The treatment with low level of As (50 mg kg^{-1}) plus Cr significantly increased Cr accumulation in roots, stems and leaves compared with the Cr treatment alone, but at high level of As (100 mg kg^{-1}) plus Cr significantly decreased Cr accumulation compared with the Cr treatment alone and low level of As plus Cr in both varieties (Fig. 2). Moreover, the treatment of As plus low of level Cr (100 and 200 mg kg^{-1}) significantly increased As level in all plant parts compared with the As treatment alone while high level of Cr (400 mg kg^{-1}) plus As significantly decreased As content compared with As alone and As plus low level of Cr treatment (Fig. 3). The effect of As and Cr concentration varied with plant organs, varieties and As, and Cr levels. The increase of As and Cr was more pronounced in O-795 than in O-9897 variety.

K^+, M^{2+} and Ca^{2+} content

The effect of Cr and As treatments on K^+, Mg^{2+} and Ca^{2+} contents in roots, stem and leaves of the two varieties are shown in Fig. 4, 5 and 6, respectively. Exposure of plants to Cr or As stresses alone resulted in significant reduction in K^+ and Mg^{2+} content in all plant parts for the both varieties except

Table 1. The effects of Arsenic and Chromium stresses on net photosynthetic rate (Pn), stomatal conductance (Gs) and transpiration rate (E) values of two jute varieties.

Variety	As level (mg kg^{-1})	Cr level (mg kg^{-1})	Pn (µmol m^{-2} s^{-1})	Gs (mol m^{-2} s^{-1})	E (m mol m^{-2} s$^{-1)}$
O-795	0	0	21.4±0.42a	0.39±0.009a	3.9±0.048a
		100	21.07±0.98a	0.38±0.009a	3.88±0.054a
		200	15.01±0.46cd	0.32±0.009bc	3.04±0.045c
		400	12.58±0.13e	0.25±0.009ef	2.53±0.012ef
	50	0	18.38±0.30b	0.34±0.009b	3.55±0.037b
		100	21.11±0.39a	0.38±0.009a	3.89±0.037a
		200	15.68±0.45c	0.33±0.008bc	3.06±0.037c
		400	12.61±0.39e	0.26±0.010e	2.57±0.037ef
	100	0	13.85±0.50de	0.28±0.010de	2.86±0.037d
		100	10.31±0.47f	0.22±0.006fg	2.66±0.037e
		200	7.85±0.44gh	0.17±0.010hi	2.19±0.037g
		400	6.77±0.48hi	0.13±0.010j	1.78±0.037h
O-9897	0	0	16.10±0.46c	0.30±0.005cd	2.93±0.045cd
		100	12.48±0.52e	0.25±0.005ef	2.49±0.072f
		200	9.97±0.60f	0.19±0.009h	2.11±0.041g
		400	7.29±0.38ghi	0.14±0.009j	1.89±0.028h
	50	0	12.75±0.44e	0.25±0.010ef	2.57±0.037ef
		100	12.68±0.43e	0.26±0.009e	2.51±0.037ef
		200	10.03±0.49f	0.20±0.010gh	2.11±0.037g
		400	7.50±0.29ghi	0.16±0.005ij	1.91±0.037h
	100	0	8.87±0.42fg	0.18±0.010hi	2.07±0.037g
		100	6.72±0.52hi	0.13±0.010j	1.79±0.037h
		200	5.67±0.46i	0.09±0.007k	1.3±0.037i
		400	3.97±0.40j	0.07±0.007k	0.98±0.037j
Interaction			**	**	**
Varity and As			**	**	**
Variety and Cr			**	NS	**
As and Cr			**	**	**
Variety + As + Cr			NS	NS	**

The same letters after the data within a column indicates that there was no significant difference ($p \leq 0.05$); * and ** indicate significance at the $p \leq 0.05$ and 0.01 level, respectively; NS = non-significant.

Table 2. The effects of Arsenic and Chromium stresses on Fe^{+++}, Mn^{++}, Cu^{++} and Zn^{++} concentration in roots of two jute varieties.

Variety	As level (mg kg^{-1})	Cr level (mg kg^{-1})	Concentration in root			
			Fe (µg g^{-1})	Mn (µg g^{-1})	Cu (µg g^{-1})	Zn (µg g^{-1})
O-795	0	0	197.42±0.62a	43.13±0.85a	22.09±0.32a	38.79±0.37b
		100	198.58±1.07a	42.15±0.64a	21.41±0.34a	38.52±0.24b
		200	136.99±1.18ef	30.52±0.55f	15.22±0.16g	28.67±0.32f
		400	106.85±1.54i	25.51±0.51g	13.82±0.11h	24.89±0.32h
	50	0	168.21±1.99b	39.64±0.74b	20.30±0.18b	37.79±0.22bc
		100	195.81±0.62a	42.88±1.04a	22.23±0.50a	38.52±0.63
		200	138.57±1.80e	31.07±0.65f	17.21±0.15e	28.38±0.50f
		400	109.64±3.36i	27.23±0.34g	16.19±0.16f	25.78±0.27h
	100	0	118.67±1.89h	36.39±0.68cd	18.00±0.38de	34.88±0.26d
		100	88.74±2.58k	27.50±0.40g	17.15±0.28e	25.85±0.26h
		200	69.06±4.13l	19.36±0.43h	15.50±0.17fg	22.91±0.38i
		400	51.07±2.18n	13.81±0.24i	14.35±0.21h	18.82±0.29k
O-9897	0	0	163.41±1.33bc	42.87±0.42a	20.10±0.26b	40.73±0.64a
		100	154.69±1.36d	33.06±0.71e	18.14±0.14d	28.20±0.30fg
		200	127.45±1.26g	25.52±0.49g	15.23±0.19g	25.22±0.11h
		400	96.43±1.56j	19.02±0.34h	12.51±0.16i	21.07±0.17j
	50	0	141.34±1.99e	37.87±0.82bc	19.18±0.19c	36.83±0.17c
		100	157.47±1.53cd	34.38±0.50de	18.24±0.15d	31.24±0.27e
		200	131.49±1.10fg	26.56±0.46g	15.58±0.20fg	27.16±0.17g
		400	98.64±1.75j	19.82±0.29h	12.69±0.20i	22.17±0.13ij
	100	0	124.78±1.12gh	35.24±0.43d	17.48±0.16de	33.74±0.24d
		100	72.51±1.89l	25.98±0.46g	15.53±0.20fg	22.12±0.17ij
		200	59.14±0.85m	19.79±0.46h	13.76±0.19h	17.76±0.14k
		400	38.59±2.36o	14.14±0.14i	11.10±0.14j	13.80±0.16l
Interaction						
Variety and As			**	**	*	NS
Variety and Cr			**	**	**	**
As and Cr			**	**	**	**
Variety + As + Cr			**	*	**	**

The same letters after the data within a column indicates that there was no significant difference ($p \leq 0.05$); * and ** indicate significance at the $p \leq 0.05$ and 0.01 level, respectively; NS = non-significant.

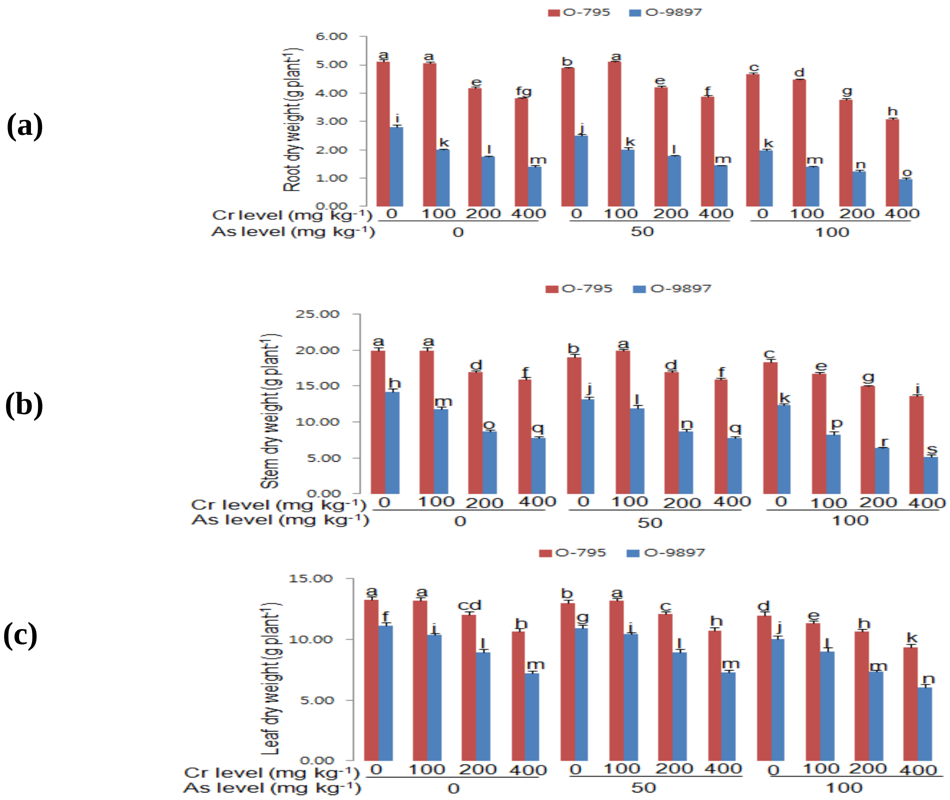

Fig 1. The effects of arsenic (As) and chromium (Cr) stresses on root (a) stem (b) and leaf dry weight (c) of two jute varieties. The same letters after the data indicates that there was no significant difference ($p \leq 0.05$).

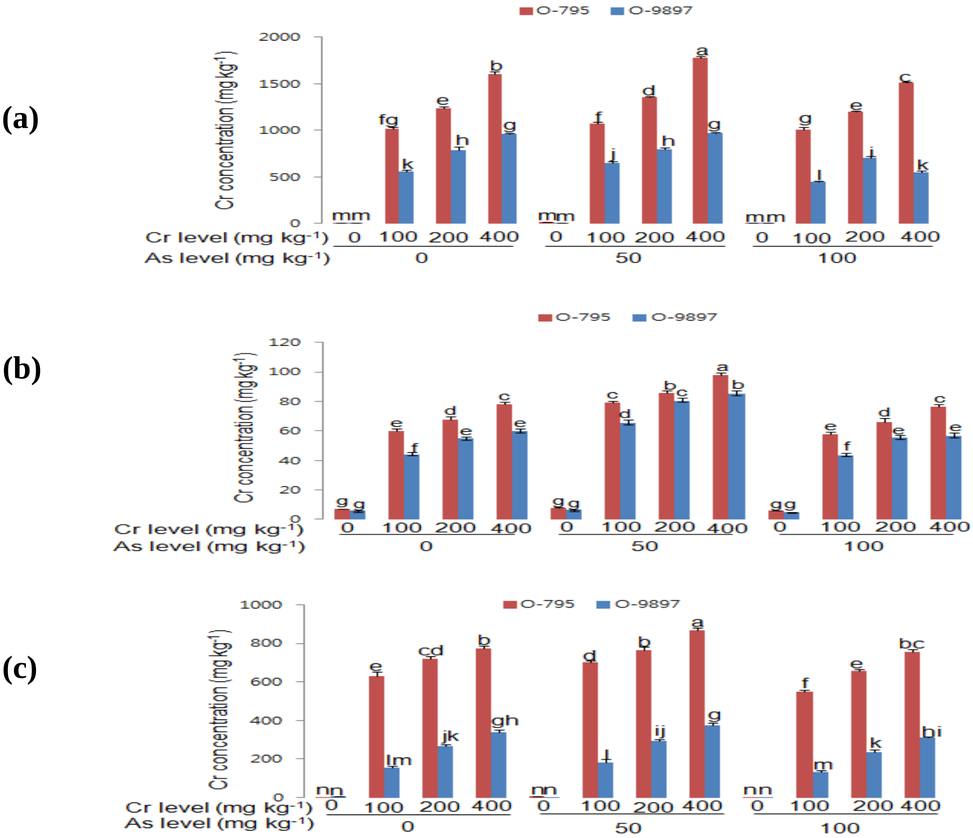

Fig 2. The effects of arsenic (As) and chromium (Cr) stresses on Cr concentration in root (a), stem (b) and leaf (c) of two jute varieties on dry weight basis. The same letters after the data indicates that there was no significant difference ($p \leq 0.05$).

except in variety O-795 at 100 mg kg^{-1} Cr where there was no significant difference between Cr treatment and the control. The treatment with low level of As (50 mg kg^{-1}) plus Cr did not decrease K^+ and Mg^{2+} concentration of all plant parts for the both varieties compared to Cr or As stress alone. On the other hand when the plants were exposed to high level As (100 mg kg^{-1}) plus Cr, the K^+ and Mg^{2+} contents were decreased significantly compared to the single stress of each metal. However, decreased K^+ and Mg^{2+} concentration was higher for O-9897 than O-795 variety. Addition of As resulted in significant increase of Ca^{2+} concentration in roots of the two varieties as compared to their controls, but stems and leaf Ca^{2+} content decreased at increased As concentration. Exposure of plants to Cr stresses caused a significant increase in Ca^{2+} content in both varieties except for variety O-795 at 100 mg kg^{-1} Cr treatment, whereas, no significant difference was found. In combined treatment, low level of As (50 mg kg^{-1}) plus Cr significantly increased Ca^{2+} content in root but stem and leaf Ca^{2+} content slightly decreased compared to single effect. Addition of high level As (100 mg kg^{-1}) plus Cr resulted dramatically increased Ca^{2+} content in root compared to single stress and low level of combined stress while stem and leaf Ca^{2+} content decreased significantly. There was a significant difference between two varieties, with Cr tolerant variety O-795 having higher Ca^{2+} content than Cr sensitive variety O-9897.

Fe^{3+}, Mn^{2+}, Cu^{2+} and Zn^{2+} content

The data for Fe^{3+}, Mn^{2+}, Cu^{2+}, and Zn^{2+} concentration in the roots stems and leaves of two jute varieties in different treatments are shown in Table 2, 3 and 4, respectively. For the Cr tolerant variety O-795, exposure of Cr significantly decreased Fe^{3+}, Mn^{2+}, Cu^{2+} and Zn^{2+} concentration in roots, stems and leaves compared to control except in Cr treatments 100 mg kg^{-1} while there was no significantly decrease in all mineral concentration between Cr treatment and control. On the other hand for the sensitive variety O-9897, these nutrient elements were decreased significantly in all Cr treatment. But, addition of As resulted significantly decreased tested nutrient uptake in all plant parts in all treatment. The As treatment was more effectively decreased all tested mineral concentration than Cr treatment. The combined treatment of low As (50 mg kg^{-1}) plus Cr slightly declined the mineral concentration compared with the Cr stress alone. However, high level As (100 mg $kg-^{1}$) plus Cr dramatically decreased all mineral concentration in all plant parts. For O-9897 variety, addition of As or Cr significantly decreased Fe^{3+}, Mn^{2+}, Cu^{2+} and Zn^{2+} concentration in roots, stems and leaves compared to control. However, low level As (50 mg kg^{-1}) plus Cr exposure slightly decreased all tested mineral concentration in all plant parts compared to the treatment of Cr alone and control. The higher level of As (100 mg kg^{-1}) plus Cr stress resulted further reduction in all nutrient contents in all plant parts. Moreover, Cr tolerant variety, O-795, had consistently lower concentration of Fe^{3+}, Mn^{2+}, Cu^{2+} and Zn^{2+} than Cr sensitive variety, O-9897.

Discussion

In addition to study of As and Cr in the soil on the uptake of mineral elements, We tested plant growth in soil pots. However, based on other earlier observations (Shi and Cai, 2009; Ali et al., 2012), in this experiment, we have also done it. As and Cr exposure can lead to numerous physiological and biochemical disorders in plants. The present study shows that As and Cr markedly reduced root, stems and leaf dry biomass except for variety O-795 at 100 mg kg^{-1}. Our result is in good agreement with previous reports showing Cr induced plant growth inhibition in ramie (Yang et al., 2010) and in radish (Tiwari et al., 2013). However, Diwan et al. (2010) reported that Cr treatment ranging from 100 to 400 mg kg^{-1} significantly enhanced the shoot and root biomass of soil-cultured Indian mustard cv. Pusa Jai Kisan. It appears that the effects of Cr on plant growth differ from plant species and culture conditions (which may affect the bioavailability). The reduction observed at higher Cr treatments could be due to impaired root growth leading to a reduced uptake of essential nutrient and water and the subsequent impact on plant biomass (Barcelo et al., 1985; Chatterjee and Chatterjee, 2000). Our results also showed that As markedly reduced root, stem and leaf biomass in all treatment in both the varieties. Similarly, reduction in biomass of plants under As stress were observed in previous studies in rice (Azizur et al., 2007), tall fescue (Jin et al., 2010), cordgrass (Mateos-Naranjo et al., 2012). The reduction of plant biomass was found more pronounced in Cr sensitive variety than Cr tolerant variety under both As and Cr stresses. Interestingly, combined exposure to low level (50 mg kg^{-1}) of As and Cr alleviated the Cr-induced inhibitory effect on dry biomass of plant compared with the Cr treatment alone (Fig. 1) indicating that low level of As plus Cr showed beneficial effect than single stress. On the other hand, we observed that the combined stress of high level of As (100 mg kg^{-1}) plus Cr caused further reduction of the measured parameters compared to the stress alone. Whereas, the more pronounced reduction was observed in Cr sensitive variety. Similarly, it has been reported that As and Cd have a synergistic effect on the inhibition of plant growth in rice (Sun et al., 2008). Short-term co-exposure of As and Cd in solution culture had synergistic effect on wheat root elongation, in contrast, As and Cd stress in a calcareous soil showed antagonistic effect (Cao et al., 2007). There was a very clear effect of As and Cr treatment on Pn, Gs and E on jute varieties except variety O-795 at 100 mg kg^{-1} Cr. Nonetheless, the decline in Pn might be attributed due to stomatal and/or non-stomatal limitations, thus As or Cr stress can affect photosynthesis in terms of CO_2 fixation, electron transport, photophosphorylation and enzyme activities (Mateos-Naranjo et al., 2012; Shanker et al., 2005). Paiva et al. (2009) reported the decrease in Pn caused by Cr (IV) probably the damage suffered by the photosynthetic system based on the decrease of the maximum quantum efficiency of PSII photochemistry (Fv/Fm). Liu et al. (2008) found that higher concentration of hexavalent Cr decreased Pn, Gs and E in *Amaranthus viridis*. It was also reported that As and Cr individually had a negative impact on photosynthetic parameter in other plants (Mateos-Naranjo et al., 2012; Subrahmanyam, 2008). In addition, Mateos-Naranjo et al. (2008) reported that Zn^{2+} entailed a simultaneous reduction in Pn and Gs in *Spartina densiflora*. The combined stress of the low As plus Cr increase photosynthetic parameter compared to Cr stress alone, but high level of As plus Cr stress showed further reduction in gas exchange parameter compared to each of these stresses alone. Moreover, O-795 had less affected than O-9897, which indicates a difference between the two jute variety in the effect of As and Cr stresses on Pn, Gs and E value. Similarly, the interaction between Al and Cd; Al and Cr can affect photosynthetic parameters more than individual metal effect (Shamsi et al., 2008, Ali et al., 2011). Most probably high level of As plus Cr interact synergistically on photosynthetic parameter (Pn, Gs and E) as

Table 3. The effects of Arsenic and Chromium stress on Fe^{+++}, Mn^{++}, Cu^{++} and Zn^{++} concentration in stems of two jute varieties.

Variety	As level (mg kg^{-1})	Cr level (mg kg^{-1})	Concentration in stem			
			Fe (µg g^{-1})	Mn (µg g^{-1})	Cu (µg g^{-1})	Zn (µg g^{-1})
O-795	0	0	93.07±1.09a	39.42±0.79a	14.15±0.33b	29.84±0.14a
		100	92.35±1.17a	38.77±1.21ab	13.74±0.18bc	29.18±0.65a
		200	71.63±1.58cde	31.27±1.00defg	10.57±0.20f	22.16±0.79fg
		400	68.06±1.04def	26.56±1.14hij	8.05±0.07i	18.16±0.21i
	50	0	85.55±2.37b	36.01±0.95abc	13.45±0.21c	26.13±0.42bc
		100	92.57±1.57a	38.47±0.81ab	13.69±0.12bc	29.51±0.52a
		200	72.73±1.37cd	31.92±1.31cdef	10.68±0.18f	22.35±0.31fg
		400	68.54±1.87de	26.76±1.05hij	8.62±0.22h	18.34±0.32i
	100	0	74.46±1.31c	31.36±1.50defg	13.18±0.17cd	23.44±0.21ef
		100	63.06±1.28fgh	27.58±1.82ghi	11.44±0.17e	20.27±0.34h
		200	53.07±1.98i	22.34±1.03kl	9.68±0.17g	16.33±0.43j
		400	43.04±1.89jk	15.41±0.98mn	5.14±0.11j	14.26±0.31k
O-9897	0	0	73.48±0.23cd	38.42±0.65ab	16.18±0.16a	27.36±0.34b
		100	66.42±1.76efg	33.14±1.36cde	12.59±0.15d	25.16±0.50cd
		200	58.25±1.24h	27.65±1.04fghi	11.09±0.12ef	21.64±0.20gh
		400	45.08±1.34j	22.96±0.84jk	7.60±0.12i	17.35±0.21ij
	50	0	69.63±1.57cde	34.88±1.06bcd	14.10±0.15b	25.78±0.27cd
		100	66.23±1.72efg	33.15±0.93cde	13.18±0.16cd	25.34±0.20cd
		200	58.68±0.88h	27.85±1.21fghi	11.33±0.09e	21.73±0.39gh
		400	45.31±0.74j	23.16±0.56jk	7.70±0.04i	17.44±0.54ij
	100	0	62.16±0.96gh	29.97±1.64efgh	13.17±0.11cd	24.33±0.46de
		100	50.82±1.13i	25.37±0.92ijk	9.45±0.23g	20.45±0.39h
		200	39.61±0.99k	18.69±0.64lm	7.78±0.07i	17.39±0.35ij
		400	21.02±0.57l	14.03±0.45n	5.01±0.05j	14.14±0.30k
Interaction						
Variety and As			*	NS	**	*
Variety and Cr			**	NS	**	**
As and Cr			**	NS	**	**
Variety + As + Cr			*	NS	**	NS

The same letters after the data within a column indicates that there was no significant difference ($p \leq 0.05$); * and ** indicate significance at the $p \leq 0.05$ and 0.01 level, respectively; NS = non-significant.

Table 4. The effects of Arsenic and Chromium stress on Fe^{+++}, Mn^{++}, Cu^{++} and Zn^{++} concentration in leaf of two jute varieties

Variety	As level (mg kg^{-1})	Cr level (mg kg^{-1})	Concentration in leafs			
			Fe (µg g^{-1})	Mn (µg g^{-1})	Cu (µg g^{-1})	Zn (µg g^{-1})
O-795	0	0	227.88±1.93a	212.46±2.25a	8.65±0.30bc	37.24±0.21a
		100	224.92±2.61a	210.24±3.03a	8.62±0.33bc	36.66±0.25a
		200	153.56±2.49d	168.66±2.69c	7.68±0.24def	27.76±0.24d
		400	136.19±3.35e	137.84±1.87e	6.18±0.19ij	20.13±0.40gh
	50	0	189.07±2.58c	191.67±2.67b	8.36±0.18cd	30.48±0.26c
		100	225.13±2.06a	211.13±2.13a	8.63±0.19bc	36.59±0.55a
		200	153.60±2.29d	169.34±2.71c	7.69±0.06def	27.78±0.23d
		400	137.05±3.00e	138.19±2.93e	6.19±0.11ij	20.41±0.23gh
	100	0	159.23±2.66d	168.68±2.99c	8.06±0.19cde	24.21±0.44f
		100	133.84±2.37e	137.68±2.49e	7.27±0.14fgh	20.52±0.30g
		200	113.35±2.52fg	116.17±3.04fg	6.64±0.16hi	16.40±0.28j
		400	91.41±3.09h	99.23±2.19h	5.43±0.16k	11.48±0.40k
O-9897	0	0	210.84±3.40b	198.12±1.61b	10.86±0.13a	35.31±0.25b
		100	183.30±2.46c	163.17±2.19c	9.31±0.13b	30.33±0.24c
		200	154.55±2.43d	144.50±3.45de	7.46±0.06efg	25.48±0.25e
		400	122.48±2.36f	109.76±2.03g	6.33±0.17ij	19.77±0.29gh
	50	0	182.88±1.93c	171.60±2.26c	9.27±0.19b	29.75±0.23c
		100	183.33±2.65c	164.64±3.18c	9.29±0.18b	30.48±0.26c
		200	154.82±2.77d	145.31±2.42de	7.43±0.14efg	25.65±0.30e
		400	122.66±2.18f	110.24±2.08g	6.33±0.21ij	19.29±0.25hi
	100	0	159.18±2.32d	152.33±2.25d	8.66±0.17bc	23.33±0.42f
		100	133.02±3.00e	123.39±2.81f	8.07±0.13cde	18.40±0.28i
		200	112.66±1.92g	97.98±2.94h	6.88±0.15ghi	15.51±0.21j
		400	81.57±2.20i	78.51±2.55i	5.79±0.11jk	9.11±0.33l
Interaction						
Variety and As			**	**	NS	*
Variety and Cr			**	**	**	**
As and Cr			**	**	*	**
Variety + As +Cr			**	*	*	**

The same letters after the data within a column indicates that there was no significant difference ($p \leq 0.05$); * and ** indicate significance at the $p \leq 0.05$ and 0.01 level, respectively; NS = non-significant.

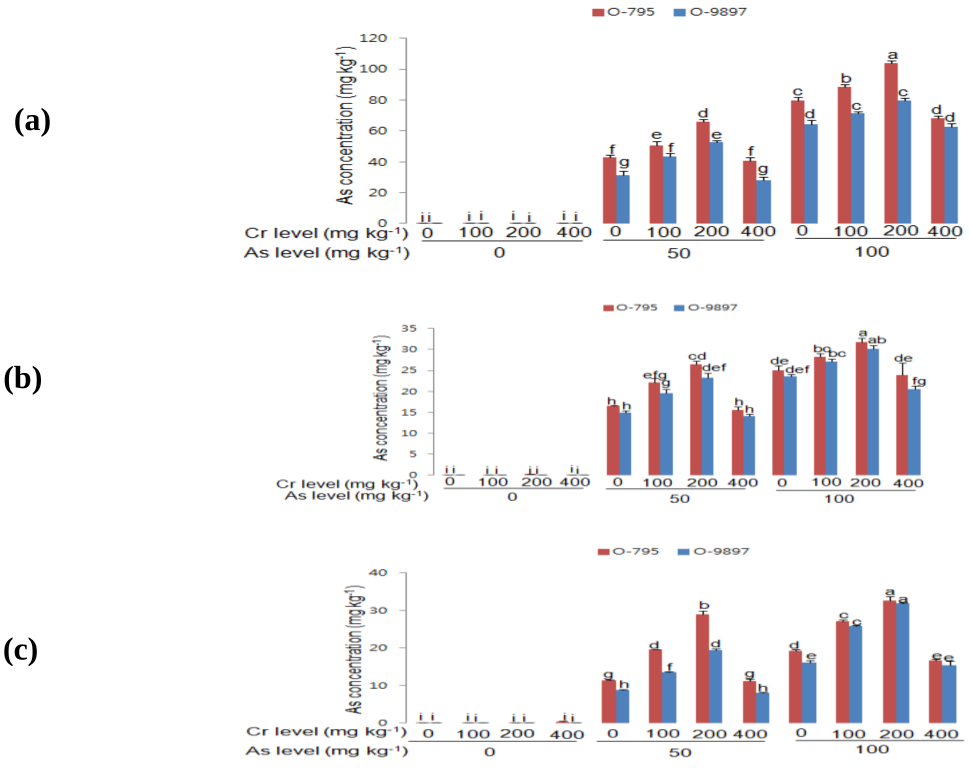

Fig 3. The effects of arsenic (As) and chromium (Cr) stresses on As concentration in root (a), stem (b) and leaf (c) of two jute varieties on dry weight basis. The same letters after the data indicates that there was no significant difference ($p \leq 0.05$).

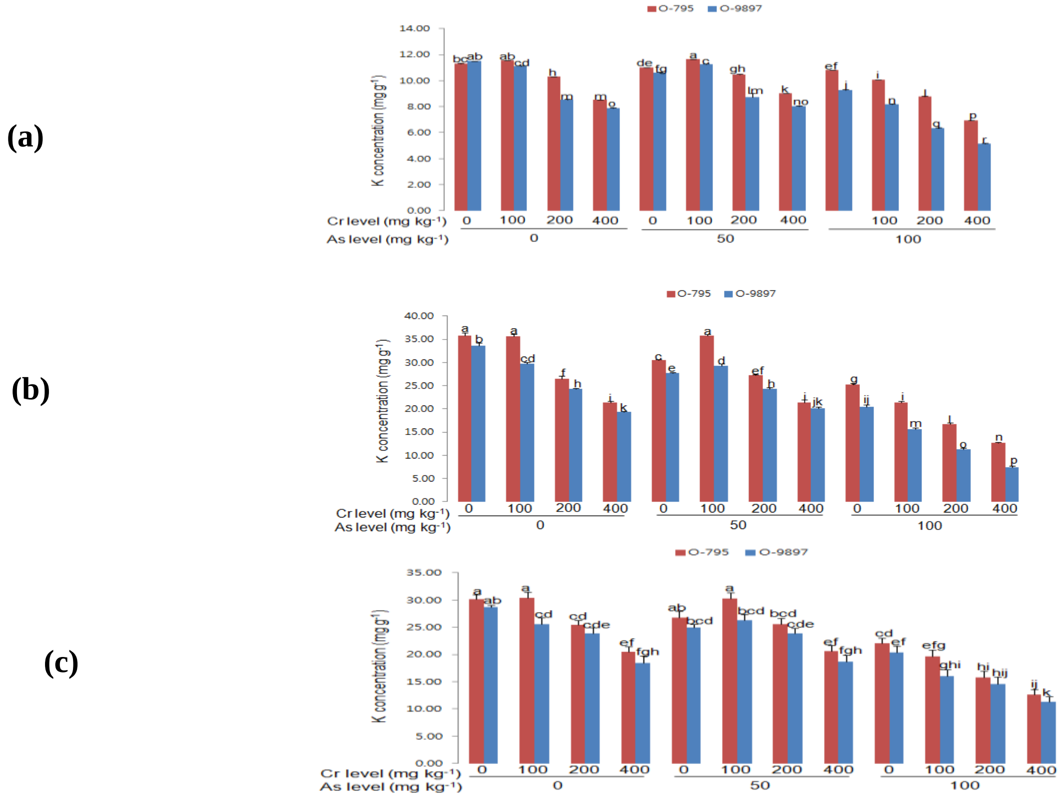

Fig 4. The effects of arsenic (As) and chromium (Cr) stresses on K^+ concentration in root (a), stem (b) and leaf (c) of two jute varieties on dry weight basis The same letters after the data indicates that there was no significant difference ($p \leq 0.05$).

observed by Ali et al. (2011). In the present study, Cr concentrations in plant tissues of the two jute varieties increased with increasing Cr level (Fig. 2). Cr accumulation in roots was considerably much higher than in the stems and leaves, indicating that most Cr absorbed in roots and a small proportion translocated into above-ground plant parts. The possible reason for Cr in roots could be the immobilization of Cr in the vacuoles of root cells or rapid reduction of Cr^{6+} to Cr^{3+} in cells. Our result showed that jute plant has similar property as of mung bean (Banerjee et al., 2008), cauliflower (Chatterjee and Chatterjee, 2000), vegetable crops (Zayed et al., 1998), Barley (Ali et al., 2011, 2012) and rice (Zeng et al., 2010). The current study showed that As concentration increased markedly with increasing As stress in plants (Fig. 3). It was observed that As was poorly translocated from root to above ground parts of jute varieties (Fig. 3), confirming the previous results in *Triticum aestivum* L. (Quanji et al., 2008), *Spartina densiflora* (Mateos-Naranjo et al., 2012). Arsenic concentration in all plant parts were relatively lower in Cr sensitive variety than that in the Cr tolerant variety and similar result were found in hyperaccumulator *Pteris vittata* (Oliveira *et al.* 2014) and rice seedling (Sun et al., 2008). The interactive effects of As with Cr on plant metal uptake can be very complex and vary with metal species (Ali et al., 2011). Interestingly, in the present study low level (50 mg kg^{-1}) of As plus Cr exposure significantly increased Cr uptake in all plant parts compared with the Cr treatment alone (Fig. 2). Thus it may be assumed that Cr and As had additive or synergetic effects on the uptake of these metals. Our results are in agreements with previously reported results, revealed that the Cd and Al had synergistic interaction on uptake of these metals in barley (Guo et al., 2007; Ali et al., 2011), and soybean (Shamsi et al., 2008). On the other hand, we observed that the combined treatment of high As level (100 mg kg^{-1}) plus Cr caused reduction of the measured parameters compared to its single stress alone. Moreover, more pronounced reduction was observed in Cr sensitive variety (Zeng et al., 2010). Zhao (2004) reported that As significantly inhibited the uptake of Cd into roots of wheat (*Triticum aestivum* L.) grown hydroponically. On the other hand, As plus low level of Cr (100, 200 mg kg^{-1}) had shown additive or synergistic effects on the uptake of As while As plus high level of Cr (400 mg kg^{-1}) showed antagonistic effect. Our results are consistent with the inability of *Pteris vittata* to reduce As in the rhizomes (Mathews et al., 2010).

Arsenic may lead to nutritional imbalance in plants due to its effect on nutrient availability, competitive uptake and transport within the plant. However, many reports were focused on the effect of As toxicity on mineral uptake, distribution and accumulation in plants (Mateos-Naranjo et al., 2012, Melo et al., 2009, Sridokchan et al., 2005). The present study investigated that As stress decreased K^+, Mg^{2+}, Fe^{3+} Mn^{2+}, Cu^{2+}, Zn^{2+} uptake and inhibited Ca^{2+} from being translocated into all plant parts in all treatment. Moreover, we observed that the extent of the inhibition differ in two varieties, with Cr tolerant variety O-795 being less affected than Cr sensitive variety O-9897. Arsenic induced reduction in nutrients in roots and shoots were probably due to As phytotoxicity. In contrast, the increased Ca^{2+} level could be a protective response to the toxicity of metals and metalloids. Calcium can stimulate the enzyme activities and can keep the cell stable (Quanji et al., 2008). Thus high level of Ca^{2+} concentration could be used to counteract As toxicity. The reduction in Mg^{2+}, Mn^{2+} and Fe^{3+} concentration in leaves with increasing As concentration could be linked to decrease in enzyme activities and photosynthetic pigments (Islam et al., 2014), due to substitution of Mg^{2+} by As in the active site (Sundaramoorthy et al., 2010). In our experiment, we observed that the decrease of Fe^{3+}, Mn^{2+} and Mg^{2+} contents in leaves in presence of As which helps to justify the reduction photosynthetic pigment concentrations. These results suggest that the activities of various enzyme and photosynthesis were decreased by arsenic. In addition, the alternation in the K^+/Ca^{2+} ratio in the guard cells and or the alternations in the abscisic acid contents which control the stomatal movement may be related to the decrease in stomatal conductance. Copper and Zn^{2+} are associated with plant transpiration. In our study, both Zn^{2+} and Cu concentrations in root, stem, and leaf were decreased with increased As concentration. Similar results were found in roots and leaves of tomato (Carbonell-Barrachina et al., 1998). Therefore, As could affect the transpiration in jute. Effects of Cr on the nutrient uptake and distribution are extremely complex and the effect varied with plant parts and Cr level (Zeng et al., 2010). The studies available on the effect of Cr toxicity on nutrient uptake in plants have provided contradicting results. According to Biddappa and Bopaiah (1989) Cr treatment markedly decreased the absorption of P, K^+, Ca^{2+}, Mg^{2+}, Fe^{3+}, Mn^{2+}, Zn^{2+} and Cu^{2+} in different parts of plant. Barcelo et al. (1985) observed that Cr decreased K^+, N, and Fe^{3+} concentrations, and enhanced Mn^{2+} and Ca^{2+} concentrations in bush bean, reduced translocation of P, Cu^{2+}, Zn^{2+} and Fe^{3+} from root to shoots. Moreover, it was reported that the effect of low level Cr (10 µM) on nutrient accumulation in rice was enhanced while at high levels Cr (50 and 100 µM) nutrient uptake and accumulation were inhibited. Chromium stress also decreased P, Ca^{2+}, and Fe^{3+} content and increased Mn^{2+} concentration in *Lolium perenne* (Vernay et al., 2007). In our study we found that high levels of Cr (200, 400 mg kg^{-1}) significantly decreased K^+, Mg^{2+}, Fe^{3+}, Zn^{2+}, Mn^{2+}, and Cu^{2+} concentration and increased Ca^{2+} concentration in both jute varieties. However, low level of Cr (100 mg kg^{-1}) stress did not significantly decrease the uptake of tested nutrients and Ca^{2+} concentration was increased in Cr tolerant variety while in case of Cr sensitive variety O-9897, it was significantly decreased, which agreed with the previous finding on rice (Zeng et al., 2008). Similarly, it has been reported that the beneficial effect of low Cr level on plant growth, yield, mineral nutrition, water relation and nutrient uptake in plant (Liu et al., 2008; Bonet et al., 1991; Han et al., 2004). However, the reduction of K^+, Mg^{2+}, Fe^{3+}, Mn^{2+}, Cu^{2+}, and Zn^{2+} might be due to the reduced root growth and impaired penetration (Fig. 1) of the roots into the soil due to Cr toxicity (Islam et al., 2014). Similar reduction trend of nutrient content was also reported in Cr stressed rice (Sundaramoorthy et al., 2010). Chromium is structurally similar with some nutrient elements like, Fe^{3+}. Cr^{6+} binds with the common carrier of Fe^{3+}, Mn^{2+}, K^+, Mg^{2+}, and therefore reduces the transport of these nutrients.

We observed that low level of As (50 mg kg^{-1}) plus Cr may alleviate the inhibiting effect of Cr on nutrient uptake in combined stress while high level of As plus Cr caused further reduction of nutrient concentration except in Ca^{2+} uptake in jute plants as compared to the individual metal stress alone. Similarly, it was reported that the interaction between Al and Cr decreased mineral nutrition in Barley (Ali et al., 2011) and Al and Cd in soybean and barley (Guo et al., 2007; Shamsi et al., 2007). Our previous results also showed that As and Cr stresses had synergistic effect on oxidative stress in jute plants (Islam et al., 2014). It may be assumed that As and Cr effect on plant nutrition might be synergetic.

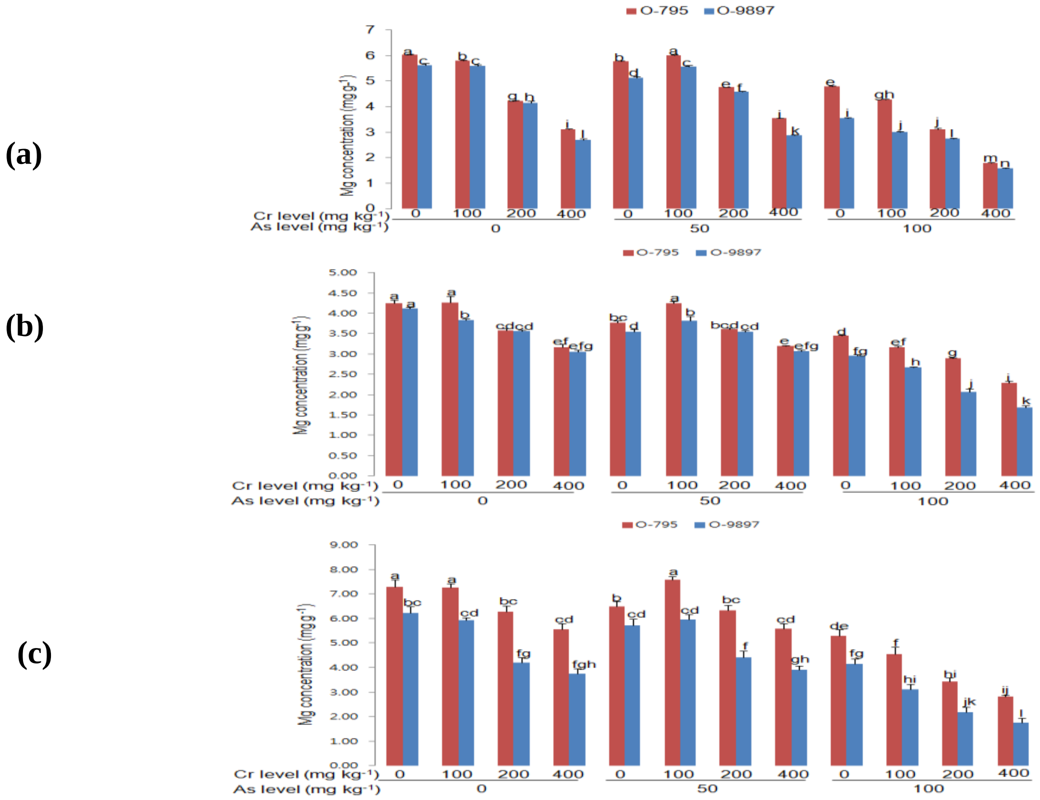

Fig 5. The effects of arsenic (As) and chromium (Cr) stresses on Mg^{++} concentration in root (a), stem (b) and leaf (c) of two jute varieties on dry weight basis. The same letters after the data indicates that there was no significant difference ($p \leq 0.05$).

Materials and Methods

Plant materials

Seeds of the two jute (*Corchorus olitorius* L.) varieties, O-795 and O-9897, were collected from Bangladesh Jute Research Institute (BJRI), Dhaka, Bangladesh.

Plant growth and treatments

The experiment was conducted in a greenhouse at the Gyeongsang National University, Jinju, South Korea (36° 50' N and 128° 26' E) during May to September 2013. Seeds of two tossa jute (*Corchorus olitorius* L.) varieties *viz.* O-795 and O-9897 differing in Cr tolerance (Islam et al., 2014) were surface sterilized in a 3% hydrogen peroxide (H_2O_2) for 5 min, rinsed with distilled water 10 times following dried on filter paper. After that seeds were directly sown into pots (45 cm×45 cm) filled with a mixture of acid-washed sand and perlite (5:4, v/v), supplemented with different amounts of potassium dichromate ($K_2Cr_2O_7$) to form 4 Cr levels (0, 100, 200 and 400 mg kg^{-1}) and sodium arsenate ($Na_3AsO_4 \cdot 12H_2O$) for As form 3 levels (0, 50 and 100 mg kg^{-1}). Thus, combination of Cr and As levels resulted in 12 treatments *viz.* As 0-Cr 0 mg kg^{-1}, As 0-Cr 100 mg kg^{-1}, As 0-Cr 200 mg kg^{-1}, As 0-Cr 400 mg kg^{-1}, As 50-Cr 0 mg kg^{-1}, As 50-Cr 100 mg kg^{-1}, As 50- Cr 200 mg kg^{-1}, As 50-Cr 400 mg kg^{-1}, As 100-Cr 0 mg kg^{-1}, As 100-Cr 100 mg kg^{-1}, As 100-Cr 200 mg kg^{-1}, As 100-Cr 400 mg kg^{-1}. The pots received natural sunlight and irrigated with drinking standard water to maintain 60% field capacity. The plants containing the pots were placed in drip trays to prevent any leachate loss. The collected leachate was returned to the respective experimental pots. At every 7th day, the each pot was fertilized with 100 ml of Hoagland's nutrient solution (H2395, Sigma, USA). As, Cr and other mineral nutrients in root, stem and leaf were analyzed.

Gas exchange measurements

Gas exchange measurements were carried out with randomly selected, fully expended leaves (n= 5 per plant). The gas exchange measurements were carried out after 60 day of after treatment. Net photosynthetic rate (Pn), stomatal conductance (Gs) and transpiration rate (E) were determined using a portable photosynthesis system under irradiance of 1200 µmol m^{-2} $s^{-1,}$ relative air humidity 60% and CO_2 concentration 500 µmol mol^{-1} of the uooermost fully expended leaf (LiCor-6400; LiCor Inc. Lincoln, Nebraska, USA) with an attached LED light source (6400-02B). The measurements were carried out from 10:00 to 12:00 am.

Growth analysis

After 60 days of seed sowing, plants were harvested and washed with tap water, followed by distilled water and deionized water three times, respectively, and then immersed into 20 mM ethylenediaminetetraacetic acid (EDTA)-Na_2 to remove metals from plant surface. After that, plant samples were separated into roots, stems and leaves, dried at 105 °C for 2 hour and then at 72 °C for 48 h in an oven until they reached constant weights. Then stem, leaf and root dry weight were measured.

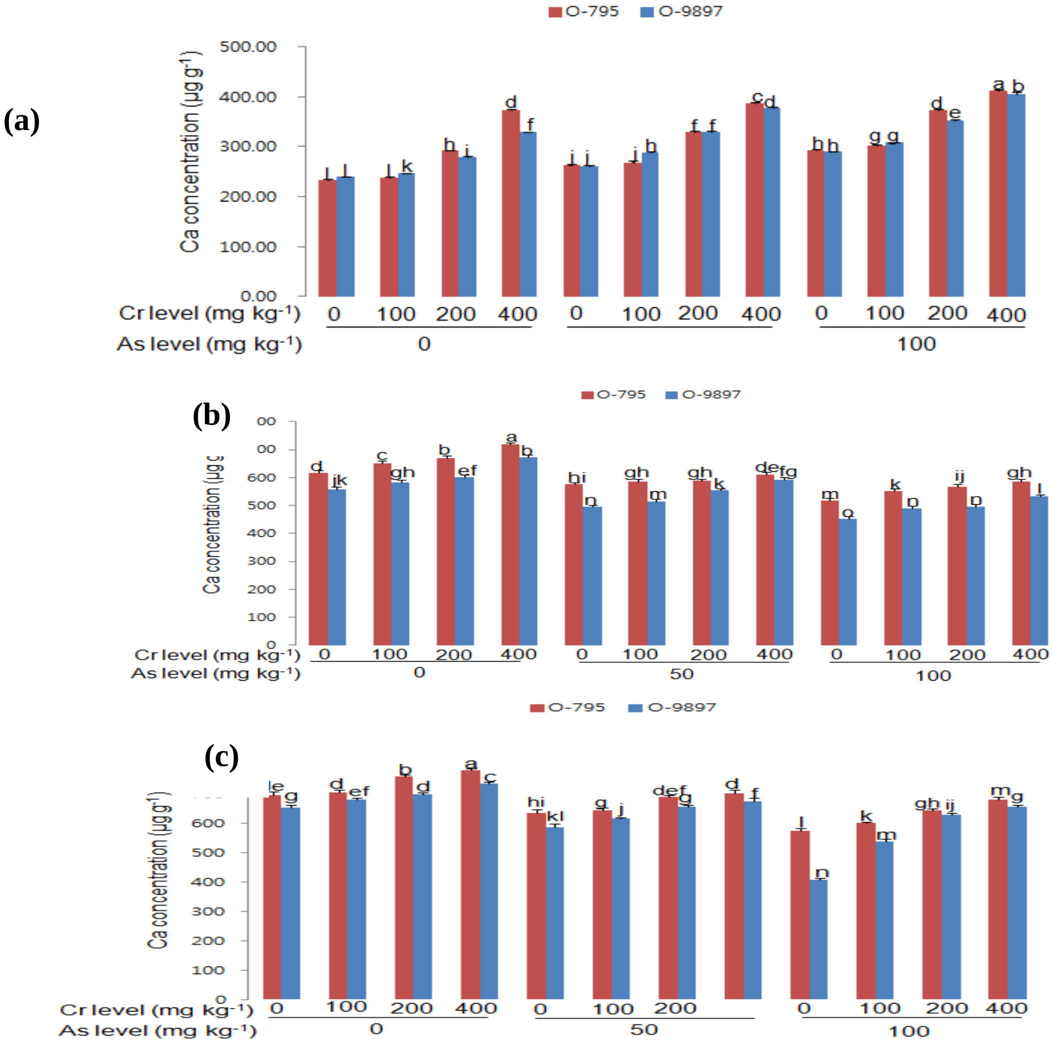

Fig 6. The effects of arsenic (As) and chromium (Cr) stresses on Ca^{++} concentration in root (a), stem (b) and leaf (c) of two jute varieties on dry weight basis. The same letters after the data indicates that there was no significant difference ($p \leq 0.05$).

Determination of metal and nutrient concentration in jute plants

The dried root, leaves and stem tissues were ground into powder using a blender (Wonder blender, Osaka chemical Co. Ltd. Japan). The 1 g of each sample was digested with HNO_3-$HClO_4$ (3:1, v/v). The contents of Cr, As and mineral elements, including K^+, Fe^{3+}, Zn^{2+}, Mn^{2+}, Mg^{2+}, Ca^{2+}, and Cu^{2+} in leaf, stem and root were analyzed by inductively coupled plasma-atomic emission spectroscopy (ICP-AES).

Statistical analysis

The experiment was arranged as a split-split plot design with jute variety as main plot, As levels as subplot, and Cr levels as sub-subplot. Each treatment had three replicates. The data were analyzed using statistical package SPSS, version 16.0 (SPSS, Chicago, IL, USA). A three-way variance analysis (ANOVA) was carried out, followed by the Duncan's multiple range tests to determine the significant difference between means of treatments.

Conclusion

In this study, we investigated the single and combined effects of As and Cr on growth, As or Cr uptake and mineral uptake by two jute varieties. Our results suggest that metal and mineral uptake by jute varieties were highly affected with high levels of As. Arsenic and Cr showed distinct interaction with each other in jute plants, which reflected on the nutrient content of plant. The combined toxicity of low level As plus Cr was less severe than of single As or Cr stress in terms of jute growth, nutrient uptake and metal uptake. Low level of As can mitigate Cr-induced inhibitory effect on plant growth but higher level As plus Cr stress result in a further reduction. Although As or Cr stress caused a dramatic reduction in nutrient contents, the combined stress of low level of As plus Cr can alleviated nutrient uptake but higher level of As plus Cr stress result in a further reduction of nutrient content in jute varieties as compared to the stress alone. Moreover, the reduced extent varied with varieties and exposure level of metal concentration. Chromium tolerant variety O-795 showed less reduction of all nutrient and metal uptake than Cr sensitive variety O-98797. In case of As and Cr interactive effect on jute, As exposure up to 50 mg kg^{-1} is supposed to be useful in mitigating Cr toxicity as As concentration more than 50 mg kg^{-1} had the synergistic effect on Cr toxicity. Low level of As plus Cr had shown additive or synergistic effect on the uptake of Cr while high level of As plus Cr showed antagonistic effect.

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