Investigating the Effectiveness of Selenite on Drought Stressed Upland Rice

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ABSTRACT

Water stress is an enormous problem facing food production, especially in arid and semi-arid regions. Production of free radicals during water stress has led to oxidative stress, which eventually causes death of cells in plants. Therefore, it is important to tackle this issue knowing that rice is one of the most important cereal crops largely cultivated and consumed by humans and animals. The studies aimed at the effect of selenite on physiological and biochemical activities of water-stressed upland rice. Three Upland rice cultivars namely Nerica U4, Nerica U7 and Vandana were collected in Africa Rice Centre, Ibadan. The seeds were sterilized and soaked for 10 hours in different concentrations of Selenite (Se) (0 mg/l, 50 mg/l and 100 mg/l). Primed seed were planted into sterilized-sieved top soils. Plants were subjected to 0 (irrigated) and 8 days (non-irrigated) water stress. Selenite 50 mg/l improved plant height, number of leaves, total carotene, chlorophyll contents, biomass, grain number of upland rice during water stress. Selenite increased activities of APX as water stress progressively increased consequently, low MDA content was observed in cultivar Vandana. Furthermore, selenite significantly improved total carotene, chlorophyll contents, anthocyanin, and dry shoot weight in cultivar Nerica U7 during water stress. Selenite significantly stabilized activities of anthocyanin and CAT in cultivar Nerica U4 during water stress. Hence high grain yield was recorded in Nerica U4 and U7 in selenite primed upland rice during water stress. Selenite reduced lipid peroxidation in upland rice at 100mg/l. Therefore, it can be concluded that response of rice to selenite during water stress is based on tolerance capacity of the cultivars and also, selenite 50mg/l can help to improve growth and yield of upland rice in drought-prone area.

Key words: Antioxidants, Growth, Lipid peroxidation, Selenite, Upland rice, Yield.

INTRODUCTION

Rice (*Oryza sativa*) is a major staple food for more than three billion people and makes up 50-80% of their daily calorie intake (Khush, 2005). Nigeria has been regarded as the largest producer of rice in West Africa sub-region (Oikeh *et al.,* 2007). Nigeria has the immanent and relevant agro-ecologies (upland, rainfed lowland, irrigated lowland, deep water and mangrove swamp) to attain self-adequacy in production of rice (Guimarães *et al.,* 2016).

Water is one of the most significant factors and the most-limiting natural resource in agriculture and food production (Wang *et al.,* 2012). Agricultural sustainability has been severely threatening due to water deficit stress causing a broad loss to agricultural production. Water stress played a crucial in rice reproductive stage, even with moderate stress; it can result in radical reduction in grain yield (Venuprasad *et al.,* 2011). Approximately 23 million hectares of rainfed rice has been affected by drought worldwide. Consequently, not only grain yield was severely affected; a lot of metabolic processes are also impaired, which eventually leads to yield reduction (Ji *et al.,* 2012). Reduction of photosynthetic activity, accumulation of organic acids and osmolytes and changes in carbohydrate metabolism are distinctive physiological and biochemical responses to drought stress (Tabaeizadeh, 1998). Water deficit also increases the formation of reactive oxygen species (ROS) resulting in lipid peroxidation, protein denaturation and nucleic acid impairment with severe consequences on overall metabolism (Hansen 2006). It is therefore required to modify crop varieties that are highly adapted to dry

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environments in order to continuously feed increasing populations with depleting water supply (Foley *et al.,* 2011).

Selenium (Se) is an essential nutrient required in low concentrations for the maintenance of animal and human health (Surai, 2006). There are evidences that Se can maintain beneficial effects on plant growth at low concentrations (Hajiboland, 2007). Selenium is known to play a crucial role in anti-oxidation in biological organisms by stimulating the activity of glutathione peroxidase, which is capable of scavenging hydrogen peroxides (Yokota *et al.,* 1988). Despite its effectiveness, its role in plant physiology and biochemical are still unclear therefore, the study aimed

to determine the effects of selenite-priming on growth and physiology of upland rice seedlings under water stress.

MATERIALS AND METHODS
Seed Source and preparation of plant material

Three upland rice cultivars namely Nerica U4, Nerica U7 and Vandana were collected from Africa Rice Centre, Ibadan, Nigeria. Cultivars Nerica U4, Nerica U7 and Vandana represented as RB, RC and RD, respectively. Air-dried sterilized seeds (30 g) were soaked for 10 hours in 50 ml of different concentrations of Sodium selenite, namely 0 (S0), 50 (S50) and 100 (S100) mg/l. The primed seeds were rinsed thoroughly with distilled water and air-dried. Two (2) sterilized, selenite-primed seeds were sown into 5 kg of sterilized sieved loamy soil. The experimental design was a completely randomized design with three replicates. Primed seeds were planted into pots arranged in the screenhouse, Department of Plant Science and Biotechnology, Federal University Oye-Ekiti, Nigeria (Latitude 7.8 ÚN and Longitude 5.21 Ú E) between April 2018 to July 2018. Four (4) weeks after planting (WAP), water stress was introduced. Non-stressed plants (D0) received 1 liter of water every day throughout the growing period while water-stressed plants were irrigated every 8 days till maturity (D8). About 1.7 liter of water was used to maintain field moisture capacity. Plants were monitored until maturity.

Data collection

Data on plant morphology were collected at maturity (12 weeks), which includes the number of leaves, number of tillers and plant height. The number of grains per plant was estimated by counting fully-filled grains after harvesting and drying to about 20% moisture content. Data was also collected on plant biomass.

Determination of Chlorophyll content

Chlorophyll was extracted from the leaves. The extraction of leaf pigments was performed with 75% acetone and the absorbance at 470, 648 and 664 nm were measured with a spectrophotometer (UV-Visible Spectrophotometer Model LT-290, Labtronics India).

Formulas for determining chlorophyll concentration (Nayek *et al.* 2004).

Chlorophyll A = $(12.25 * A664nm) - (2.79 * A646.8)$
Chlorophyll B = $(21.5 * A648nm) - (5.1 * A664)$
Total Carotene= $(1000 * A470 - 1.82C_A - 85.02C_B)/198$

Biochemical determination

Leaf samples were homogenized in cold 50 mM Sodium phosphate buffer (pH 7.8) for the enzyme extractions. Homogenate was centrifuged at 12,0009g for 15 min at 4°C. Ascorbate peroxidase (APX) (Nakano and Asada, 1981) Catalase (CAT) (Aebi, 1984) activities were measured. The total amount of lipid peroxidation products present in the plant samples was estimated by the thiobarbituric acid (TBA) method which measures the malondialdehyde (MDA) reactive products (Ohkawa *et al.,* 1979). The total anthocyanin content of the extract was determined by the pH differential method (Wrolstad *et al.,* 2005).

RESULTS AND DISCUSSION

Water stress significantly reduced the plant height ($p < 0.05$) and the number of leaves ($p < 0.01$) during water stress (Fig 1A, 1B). All concentration (S50 and S100) of selenite used significantly ($p < 0.05$) increased the plant height when compared to S0 and the highest plant height was recorded under selenite S50 (Fig 1A). On the contrary, selenite had no effect on number of leaves and tillers of upland rice with or without water stress (Table 1; Fig 1B). The number of tillers was not sensitive to water stress. Growth and yield of rice were reduced when water stress persisted more than 7-14 days (Manish *et al.*, 2018). However, plant height and number of leaves were increased in all selenite primed rice cultivars during water stress. Furthermore, rice seed primed with 90 and 105 µmol L⁻¹ selenium solutions showed a retarded growth (Khaliq *et al.,* 2015).

Table 1: Mean square values of plant height, number of leaves and number of tillers of selenite-primed upland rice seeds under water stress.

SV	DF	Mean square		
		PH	NL	NTil
Cultivars	2	29.50ns	1.13ns	0.60ns
Selenium	2	38.57*	0.54ns	0.51ns
Drought levels	1	51.51*	17.01***	8.00ns
Cultivars*Selenium	4	6.02ns	0.35ns	0.58ns
Cultivars*Drought levels	2	3.71ns	1.26ns	0.54ns
Selenium*Drought levels	2	2.50ns	0.35ns	0.54ns
Cultivars*Selenium*Drought levels	4	7.63ns	0.16ns	0.02ns
Error	54	10.47	0.54ns	0.46ns
Total	71			
R-sq (%)		0.31	0.47	0.37
CV		61.63	6.29	2.94

SV: source of variation; DF: degree of freedom; R-sq: R-square; CV: coefficient of variation; PH: plant height; NL: number of leaves; NTil: number of tillers; *significant at $p < 0.05$; ***significant at $p < 0.01$; ns: not significant.

Cultivars, selenite and water stress had a significant (p<0.001) effect on the total carotene and chlorophyll contents of the upland rice (Table 2). There was an increased in chlorophyll A content at S50 when there was no water stress (D0). During water stress, Nerica U7 had the highest chlorophyll A content at S50 (Table 3). Cultivar Nerica U7

had the highest chlorophyll B with (D8) or without water stress (D0) at S50. The highest total carotene was recorded in cultivar Nerica U7 at S50 when there was no water stress (DO). However, when water stress was imposed, Nerica U4 at the highest total carotene content at S50 (Table 3). The lowest total carotene and chlorophyll contents were recorded
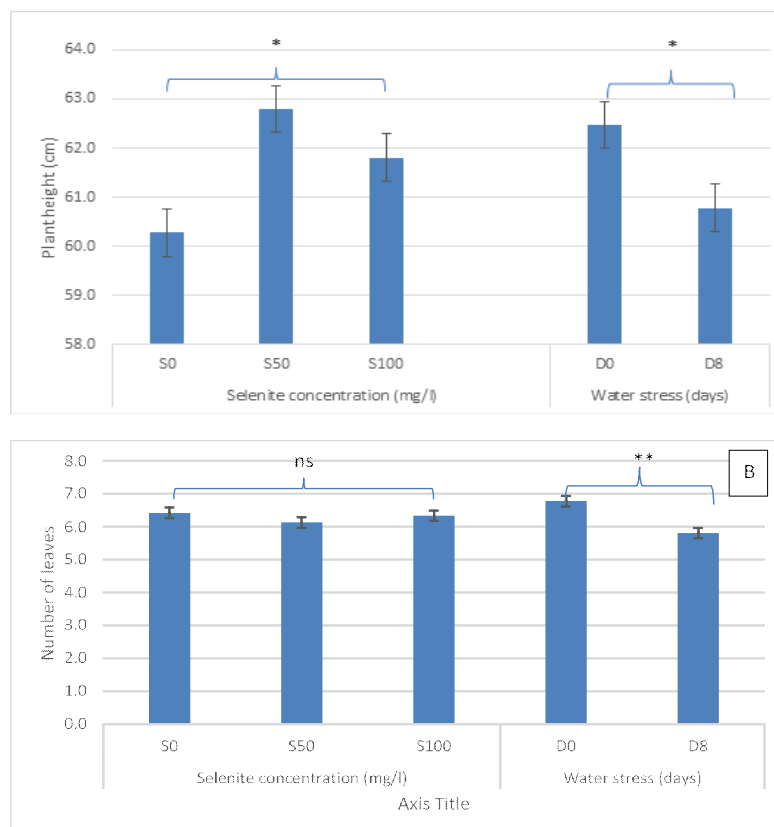
Fig 1: (A) Effect of selenite priming (mg/l) and water stress on plant height (cm) and (B) number of leaves of upland rice. S0= 0g/ml Selenite, S50=50 mg/l Selenite, S100 Selenite =100 mg/l Selenite, D0= well-watered, D8= 8days non-irrigated. Bars represent means separated with error bars mean±standard error.

Table 2: Mean square values of physiological parameters generated by the analysis of variance of selenite-primed upland rice seeds subjected to water stress.

SV	DF	Mean square						
		Antho	MDA	CAT	APX	Total carotene	ChlA	ChlB
Cultivar	2	79.78***	0***	0.047ns	0.000008*	61.12***	90.66***	345.20***
Drought level	1	0.007ns	0***	0.049ns	0.000011*	11.63***	13.62***	26.33***
Selenium	2	36.00**	0***	0.038ns	0.000005ns	30.43***	54.95***	229.31***
Cultivar*Water stress	2	12.84ns	0***	0.109	0.000004ns	8.99***	15.02***	206.42***
Cultivar*Selenium	4	33.32**	0***	0.031ns	0.000006ns	19.56***	45.57***	189.25***
Water stress*Selenium	2	60.54***	0ns	0.004ns	0.000009*	22.47***	17.31***	81.96***
Cultivar*Water stress*Selenium	4	204.25***	0***	0.049ns	0.000012**	72.64***	17.33***	86.98***
Error	18	5.908	0.00	0.025	0.000002	0.48	0.05	0.57
Total	35							
R-sq		0.93	0.99	0.62	0.77	0.99	0.99	0.99
CV		74.89	0.000076	0.2	0.0014	17.85	12.51	34

SV: source of variation; DF: degree of freedom; R-sq: R-square; CV: coefficient of variation. *significant at p<0.05; ***significant at p<0.01; ns: not significant; Antho: anthocyanin; MDA: malondialdehyde; CAT: catalase; APX: ascorbate peroxidase; ChlA: chlorophyll A; ChlB: Chlorophyll B.

in cultivar Vandana (Table 3). All cultivars without selenite had a high total carotene as the drought stress increased. Selenite concentration at 50 mg/l significantly increased the total carotene content and chlorophyll contents of all the cultivars. Unlike cultivars without selenite, chlorophyll contents were directly affected by water stress. Negative effects were observed on the photosynthetic rate and chlorophyll fluorescence of *Phaseolus vulgaris* cultivated under drought stress (Zadražnik *et al.*, 2020). Each cultivar also varied in the total carotene content when primed with selenite under water stress conditions. Selenite increased the chlorophyll content A and B of cultivar Nerica U7 under water stress. High anthocyanin in selenite primed Nerica U7 could be responsible for high chlorophyll contents during water stress which might be as a result of the tolerant ability of the cultivar. Genetic variability and differential respond observed in plants could be due to their genotypes (Nkoana *et al.*, 2019).

Table 3: Responses of chlorophyll A, chlorophyll B and total carotene to the interaction of upland rice cultivars, selenite priming concentration and water stress levels. Means are separated using Duncan Multiple Range Test from the Analysis of variance.

Parameter	Water stress level	Selenite concentration	Upland rice cultivars		
			RB	RC	RD
Chlorophyll A µg/ml	D0	S0	8.0d	15.7b	10.1a
		S50	13.0bc	20.9a	11.2a
		S100	11.5c	9.1c	7.6b
	D8	S0	13.7b	8.7c	10.8a
		S50	14.3ab	22.7a	7.8b
		S100	15.9a	13.7b	10.5a
Chlorophyll B µg/ml	D0	S0	26.4c	38.9b	30.8a
		S50	34.8b	55.8a	29.5a
		S100	23.8c	32.4b	25.9c
	D8	S0	44.8a	21.1c	28.5b
		S50	33.7b	50.2a	30.2a
		S100	38.5b	38.7b	28.0b
Total carotene µg/ml	D0	S0	13.6bc	16.8b	17.1b
		S50	13.7bc	31.1a	19.2a
		S100	22.4a	18.2b	13.6c
	D8	S0	15.8b	19.6b	18.0a
		S50	21.2a	18.4b	14.6c
		S100	12.4c	18.7b	17.0b

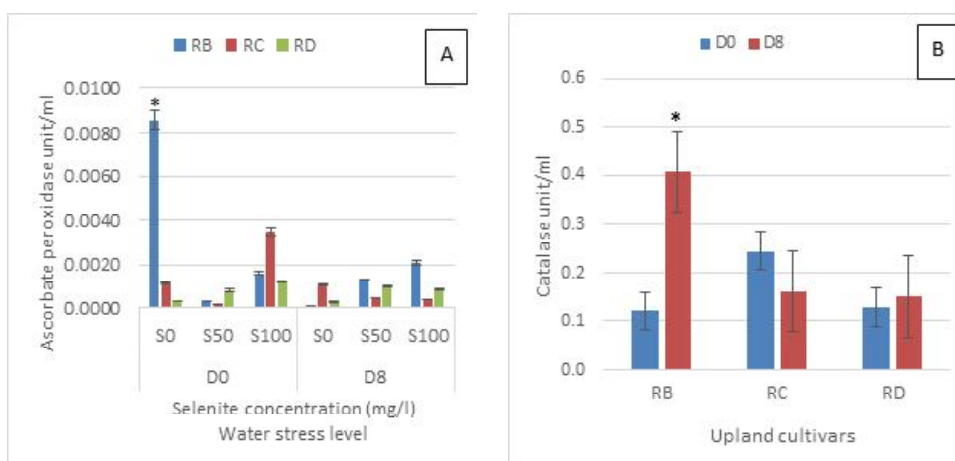Means along the row with the same alphabet are not significantly different.
S0= 0g/ml Selenite, S50=50 mg/l Selenite, S100 Selenite =100 mg/l Selenite, D0= well-watered, D8= 8days non-irrigated, RB= Nerica U4 RC= Nerica U7 RD= Vandana.

Table 4: Activities of Anthocyanin and MDA contents of selenite primed upland rice under water stress.

Cultivar	Selenium	Anthocyanin (µg/l)		MDA (M)	
		D0	D8	D0	D8
RB	S0	79.24a	68.38b	0.000095a	0.000095a
	S50	75.14a	79.32a	0.000107a	0.000110a
	S100	75.98a	80.99a	0.000095a	0.000072b
RC	S0	85.16a	70.39b	0.000060b	0.000083a
	S50	72.47a	79.90a	0.000058b	0.000072a
	S100	67.71b	81.82a	0.000077b	0.000136a
RD	S0	61.87b	72.31a	0.000051a	0.000047b
	S50	74.56a	73.64a	0.000057b	0.000068a
	S100	81.99a	67.13b	0.000062a	0.000039b

Means along the row with the same alphabet are not significantly different.
S0= 0g/ml Selenite, S50=50 mg/l Selenite, S100 Selenite =100 mg/l Selenite, D0= well-watered, D8= 8days non-irrigated, RB= Nerica U4 RC= Nerica U7 RD= Vandana.

Fig 2: Variations in ascorbate peroxidase (APX) (A) and catalase (CAT) (B) activities of selenite-primed upland rice seeds under water stress. S0= 0g/ml Selenite, S50=50 mg/l Selenite, S100 Selenite =100 mg/l Selenite, D0= well-watered, D8= 8days non-irrigated, RB= Nerica U4 RC= Nerica U7 RD= Vandana. Bars represent means separated with error bars mean±standard error.
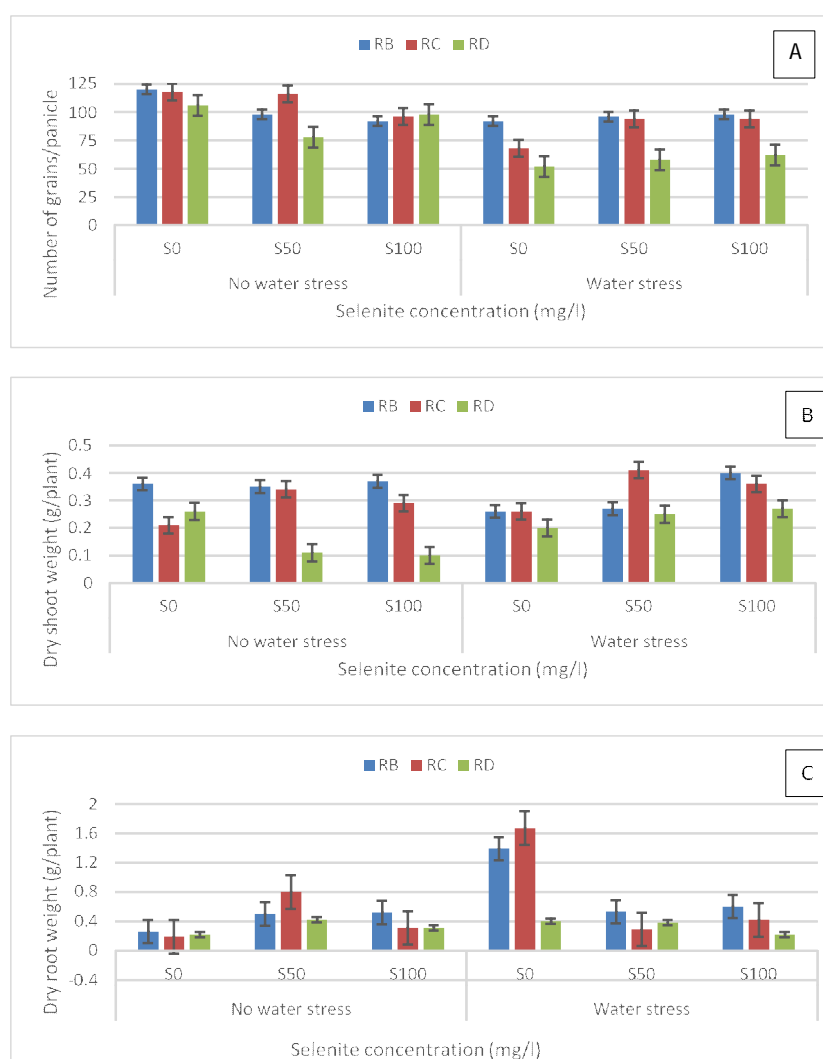
Fig 3: Number of grains, dry root weight (g) and dry shoot weight (g) of selenite-primed Upland Rice subjected to water stress. S0= 0g/ml Selenite, S50=50 mg/l Selenite, S100 Selenite =50 mg/l Selenite, D0= well-watered, D8= 8days non-irrigated. Error bars are set at mean±SE.

Selenite had no significant effect on APX with or without water stress except for cultivar Nerica U4 and Nerica U7 at S0 and S100 respectively, which were significantly ($p<0.05$) high (Fig 2A). Selenite increased the activity of CAT in cultivars Nerica U4 and Vandana during water stress (D8) when compared to plants without water stress. However, cultivar Vandana had the lowest CAT activities (Fig 2B). Selenite increased APX and CAT with or without water stress in cultivar Nerica U4 than other cultivars. Antioxidant enzymes were enhanced in selenite primed seeds (Yokota, 1988). Similarly, enzyme CAT had been boosted in selenite treated wheat plants when exposed to abiotic stress (Agbolade *et al.,* 2019). Other cultivars showed no significant differences in their APX and CAT with or without water stress. Despite an increase in anthocyanin during water stress, Ascorbate peroxidase APX activity of cultivar Nerica U7 was not significant thus high MDA contents were observed in this cultivar. Low Selenite induced levels of Peroxidase (POD), whereas APX activity decreased (Cartes *et al.,* 2009). In spite of this non-significant effect of selenite on number of leaves, tillers APX and chlorophyll contents of cultivar Nerica U4, it was interesting that anthocyanin and catalase activity increased significantly under water stress, this may be an indication for mild tolerance to water stress (Fig 2 and Table 4). Ascorbate peroxidase (APX) was significantly ($p<0.05$) affected by cultivar and water stress but not by selenite primed concentrations. However, each cultivar behaved differently in its APX activities under water stress and selenite priming treatment. APX plays a key role in catalyzing the process of detoxifying hydrogen peroxide in plant cells under stressful environmental conditions (Caverzan *et al.,* 2012). Higher levels of APX production in plants have been attributed to their tolerance to water stress (Kong *et al.,* 2016). However, APX activities in the cultivars used in this study were not significantly higher under water stress conditions compared to non-stress conditions, except for cultivar Nerica U4 and Nerica U7.

Though low total anthocyanin was observed in cultivar Vandana however, an increased in APX with low MDA content was recorded as selenite concentration increase during water stress (Tables 2 and 4). Similarly, the rice seedlings emerged from primed seeds manifested significantly lower MDA contents as well as ROIs-accumulation (Hussain *et al.,* 2016). No significant differences in the shoot POD activity were observed among plants grown with Se supply levels from 0 to 2 µM, except at 1 µM Se (Kong *et al.,* 2016).

During water stress, the grain number per panicle ranged from Nerica U4>Nerica U7>Vandana in all the concentrations of selenite. Nerica U4 and U7 had the highest grain number per panicle when there was no water stress (D0) at S0 (Fig 3A). Nerica U4 at S50 and S100 was high though not significant with the grain number per panicle in cultivar Nerica U7 at S50 and S100. During water stress, the number of grain per panicle increased as concentration of selenite increase in cultivars Nerica U7 and Vandana

(Fig 3A). During water stress (D8), Nerica U7 had the highest dry shoot and root per plant at S50 and S0 respectively (Fig 3B and 3C). There was no significant difference in dry root of all the cultivars except Nerica U4 and U7 at S0 (Fig 3C). At S100, the dry shoot ranged from Nerica U4>Nerica U7>Vandana with or without water stress (Fig 3B).

This showed that selenite improved the number of grains per panicle under water stress conditions in cultivar Nerica U4 and Nerica U7. However, selenite priming did not have any effect on number of grain in cultivar Vandana. It was also observed that dry shoot weight was improved in all three (3) cultivars with selenite treatments under water stress conditions. Selenite 50mg/l increased the dry shoot weight of Nerica U7. Selenium did not improve biomass in wheat under normal watering condition but there was a significant increase in biomass with selenium treatment under water stress condition (Nawaz *et al.,* 2013). Similarly, an improved yield in selenite primed wheat plants under abiotic stress conditions was observed (David *et al.,* 2018).

CONCLUSION

Selenite concentration of 50mg/l increased the total carotene, chlorophyll contents, grain yield and biomass of the upland rice and selenite 100 mg/l has the ability to lowered MDA content of upland rice during water stress. Also, selenite increased enzyme APX and total anthocyanin which were responsible for significant reduction of MDA contents. Moreover, the vegetative and reproductive abilities of upland rice under water stress were also based on the tolerance abilities of the cultivars hence; farmers are also encouraged to cultivate upland rice treated with 50mg/l of selenite in drought-prone area.

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